

2

Background to manufacturing practice

2.1 Introduction

The process of yoghurt making is an ancient craft which dates back thousands of years and possibly even to the domestication of the cow, sheep or goat, but it is safe to assume that prior to the nineteenth century the various stages involved in the production of yoghurt were little understood. The survival of the process through the ages can be attributed, therefore, to the fact that the scale of manufacture was relatively small, and hence the craft was handed down from parents to children. However, over the last few decades the process has become more rational, mainly due to various discoveries and/or improvements in such disciplines as:

- microbiology and enzymology
- physics and engineering
- chemistry and biochemistry.

Yet by today's standards of industrial technology, the process of yoghurt making is still a complex process which combines both art and science together.

The micro-organisms of the yoghurt starter cultures play an important role during the production of yoghurt, for example, the development of acid and flavour. Their classification, behaviour and characteristics are discussed in detail in Chapter 7. However, in order to understand the principles of yoghurt making, it will be useful to describe separately the various stages of manufacture and their consequent effects on the quality of yoghurt. The technology of the process, that is, the equipment required for small and large scale production, will be discussed in Chapter 3.

The traditional and the improved methods for the manufacture of yoghurt are illustrated in Fig. 2.1. It can be observed that the former process has several drawbacks, such as:

- Successive inoculations of the starter culture tend to upset the ratio between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, or may lead to mutation beyond the 15–20th subculturing.

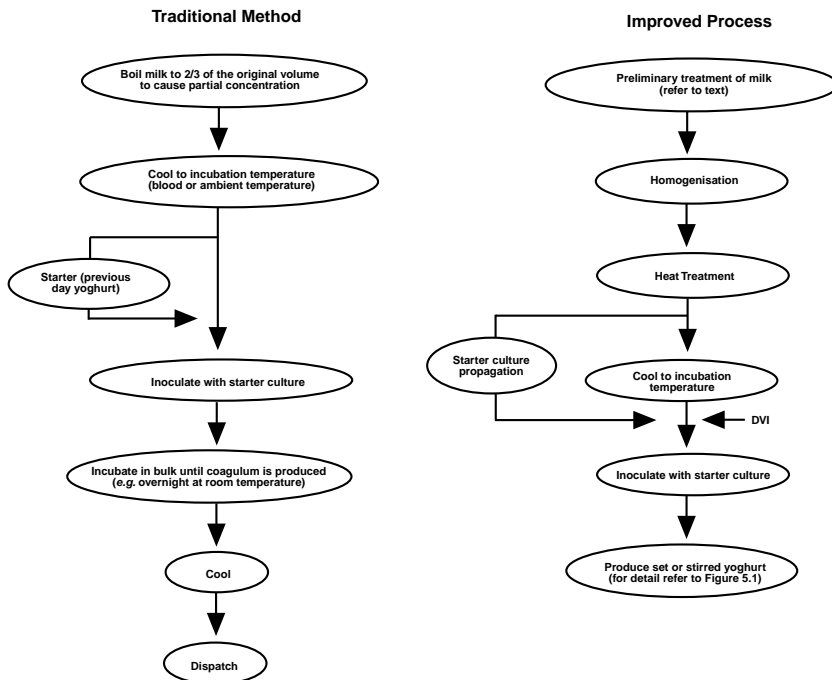


Fig. 2.1 Generalised scheme illustrating the different methods for the production of yoghurt

- The low incubation temperature, for example, ambient, results in slow acidification of the milk (18 hours or more), compared with the optimum conditions of 40–45°C for 2½–3 hours.
- The slow rate of acid development may promote undesirable side effects, for example, whey syneresis, which can adversely affect the quality of yoghurt.
- The traditional process provides no control over the level of lactic acid produced during the fermentation stage.

Nevertheless, despite these drawbacks it is obvious that the traditional process has laid the basic foundation for the production of yoghurt as practised in the industry at the present time (see Fig. 2.1). In reality, the basic changes depend on the following:

- the purity of the yoghurt starter cultures which can be obtained from commercial starter manufacturers, starter banks or research establishments;
- the ability of dairies to propagate these cultures in sterile milk under aseptic conditions, so giving rise to active reliable starters; however, at present direct-to-vat inoculation (DVI) of the starter culture is widely used;
- the temperature of incubation can be accurately controlled, so that the rate of acid development and the processing time is known in advance;
- the cooling of the yoghurt can be carried out quickly at the desired level of acidity, and the quality of yoghurt is more uniform;

- the development of easy methods for measuring the rate of acid development in milk (using pH meters and/or acidimeters) enables even a semi-skilled operator to control the process adequately.

2.2 Preliminary treatment of the milk base

The bulk chemical composition of milk is mainly of water, but it also contains a mixture of complex components such as proteins, carbohydrate, fats, minerals and vitamins which are the main source of food for the young mammal. A detailed breakdown of these components is shown in Fig. 2.2. The characteristics of each chemical component have been discussed elsewhere in detail and the reader is

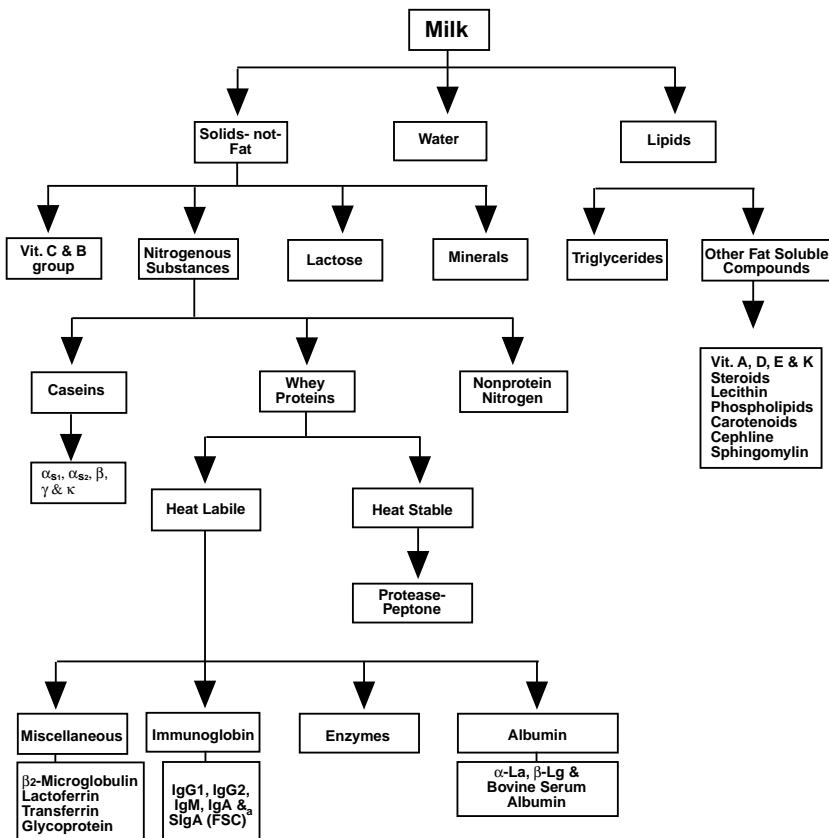


Fig. 2.2 Typical example of the main chemical components of cow's milk

^a IgA could be also associated with another secretory component and the complex may occur in a free state.

Note: The milk also contains dissolved gases (O_2 , CO_2 and N_2), enzymes (lipases, reductases, proteases, phosphatases, lactoperoxidases, catalases, oxidases, etc.), cellular matter (epithelial cells, leucocytes), micro-organisms (bacteria, yeasts and moulds) and contaminants due to carelessness during milking (straw, leaves, soil, disinfectant, etc.).

Adapted from Ling *et al.* (1961), Larson and Smith (1974c), Walstra and Jenness (1984), and Scott (1986).

referred to some reviews for a more complete discussion (Fox, 1992, 1994, 1997; Jakob, 1994; Pearce, 1995; Swaisgood, 1996).

2.2.1 Milk as a raw material

Milks of different species of mammals have been used for the production of yoghurt. Table 2.1 illustrates the major differences in the chemical composition of these milks. As a result, variations in the quality of yoghurt do occur, depending on the type of milk used. For example, milk containing a high percentage of fat (sheep, buffalo and reindeer) produces a rich and creamy yoghurt with an excellent “mouth-feel” compared with yoghurt manufactured from milk containing a low level of fat, or milk deprived of its fat content, for example skimmed milk. The lactose in milk provides the energy source for the yoghurt starter organisms, but the protein plays an important role in the formation of the coagulum and hence the consistency/viscosity of the product is directly proportional to the level of protein present; yoghurt produced from unfortified mare’s and ass’s milk would be less viscous than yoghurt made from sheep’s or reindeer’s milk.

Although the flavour of yoghurt is mainly the result of complex biochemical reactions initiated by microbial activity, the flavour of the milk base varies from species to species and this characteristic is reflected in the end product.

Since cow’s milk is widely available in most countries around the world, the emphasis will be on the use of this type of milk for the manufacture of yoghurt, although even when considering cow’s milk, there are quite large differences in composition (Table 2.2). The major constituents of milk are: water, fat, protein, lactose and minerals (ash). A detailed breakdown of these components is shown in Fig. 2.2.

Inevitably, the chemical composition of fresh milk varies from day to day within any particular breed depending on such factors as stage of lactation and age of the cow, milk intervals, season of the year and environmental temperature, breed of cows and breeding policy, efficiency and intervals between milking, nutrition, hormones and/or disease of the udder. The following are recommended for further reading regarding aspects of dairy cow husbandry (Larson and Smith, 1974a, b, c; Larson, 1978; Phillips, 1996). Figure 2.3 illustrates the monthly variations in the fat

Table 2.1 Chemical composition (g 100 g⁻¹) of milk of different species of mammals

Species	Water	Fat	Protein	Lactose	Ash
Ass	89.0	2.5	2.0	6.0	0.5
Buffalo	82.1	8.0	4.2	4.9	0.8
Camel	87.1	4.2	3.7	4.1	0.9
Cow	87.4	3.9	3.3	4.7	0.7
Goat	87.0	4.5	3.3	4.6	0.6
Horse	88.8	1.9	2.6	6.2	0.5
Reindeer	63.3	22.5	10.3	2.5	1.4
Sheep	81.6	7.5	5.6	4.4	0.9
Yak	82.7	6.5	5.3	4.6	0.9
Zebu	86.5	4.8	3.3	4.7	0.7

Adapted from Lentner (1981), Jenness (1988) and Holland *et al.* (1991).

Table 2.2 Commercial (average expected) composition of cow's milk ($\text{g } 100 \text{ g}^{-1}$)

Breed	Fat	Protein	Lactose	Ash
Ayrshire	3.85	3.35	4.95	0.69
Friesian	3.40	3.15	4.60	0.73
Guernsey	4.90	3.85	4.95	0.75
Jersey	5.14	3.80	5.00	0.75
Shorthorn	3.65	3.30	4.80	0.69

After Scott (1986).

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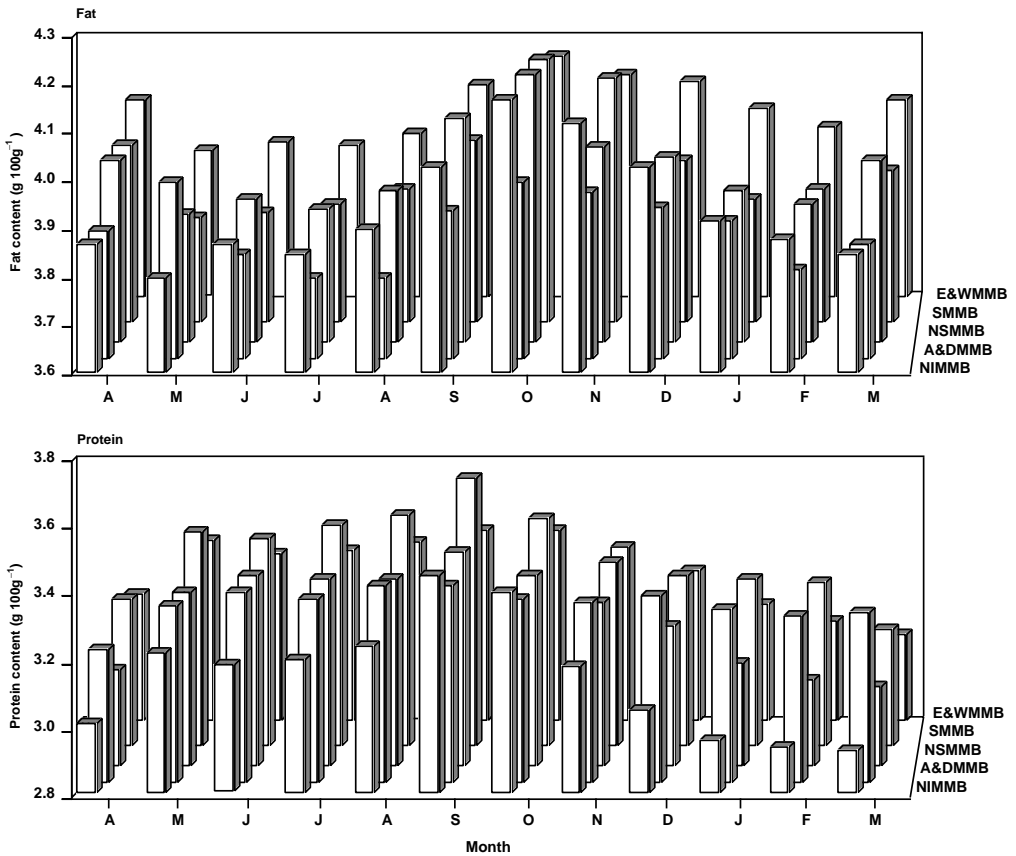


Fig. 2.3 Monthly variation ($\text{g } 100 \text{ g}^{-1}$) of the fat and protein contents of milk obtained from the former Milk Marketing Boards in the U.K.

Since the ash and lactose contents in bulk milk vary little, figures of 0.75 and $4.5 \text{ g } 100 \text{ g}^{-1}$, respectively, are taken as an annual averages. The data were obtained between April 1993 and March 1994, before these schemes were revoked on 31 October 1994.

England & Wales Milk Marketing Board (E&WMMB), Scottish Milk Marketing Board (SMMB), North of Scotland Milk Marketing Board (NSMMB), Aberdeen & District Milk Marketing Board (A&DMMB), and Northern Ireland Milk Marketing Board (NIMMB).

Data compiled from Pickett (1996).

and protein contents of milk from the former Milk Marketing Board regions in 1993–94 before these schemes were revoked on 31 October 1994 (Pickett, 1996). In order to overcome these inherent variations in composition, fresh liquid milk has to be standardised and/or fortified:

- to comply with existing or proposed legal standards for yoghurt, that is, the percentage of fat and/or solids-not-fat (see Chapter 10);
- to standardise the quality of yoghurt, that is, acidity, sweetness and consistency/viscosity of the coagulum to meet the demands of the consumer; the former two factors can be controlled during the production stages, but the consistency/viscosity of yoghurt is affected by the level of protein present in the milk and hence fortification of the milk solids-not-fat fraction is of primary importance.

2.2.2 Separation of cellular matter and other contaminants present in milk

Liquid milk may contain cellular material, for example, epithelial cells and leucocytes, which originates from the udder of the cow, and is, in some instances, due to carelessness during milk production. The milk is prone to further contamination with straw, leaves, hair, seeds, soil, etc. The primary objective of a milk processor is to remove such contaminants from the milk in order to ensure a better quality end product and while different methods are employed in dairies, the most universal system is the cloth filter. However, this method of filtration does have its limitations, one of which is that it can only remove the large debris present in the milk.

During the manufacture of some varieties of cheese, the presence of spore-forming organisms and/or cellular matter can affect the quality of the product and since the level of heat treatment of the cheese milk is limited to 72°C for 15s, survival of the spores can lead to product loss.

Centrifugal clarification has been employed, with limited success, to remove spores, but unfortunately the treatment tends to break the bacterial clumps and the milk sours more quickly. However, the principle of centrifugation has been exploited in a high speed separator known as a bactofuge and this type of separator can remove many undesirable micro-organisms from milk together with a very small amount of the milk constituents. In practice, the separated fraction (bactofugate) amounts to around 2–3% of the total throughput of milk. The bactofuge is then subjected to a sterilisation treatment by live steam injection at 130–140°C for 3–4s and, after cooling, is added back into the pasteurised cheese milk. Another method of removing spore formers from cheese milk known as microfiltration has become available. Details and illustrations of this method of processing have been reported by Tamime (1993). The application of this high heat treatment to only a small portion of the milk overcomes the problems associated with the presence of spore-forming organisms and at the same time does not affect the quality of the cheese.

However, the use of bactofuge separators or microfiltration on a yoghurt processing line is not really necessary since the heat treatment of the milk base (see Section 2.9.1) is high enough to eliminate, or at least reduce drastically, the undesirable organisms in the yoghurt milk and, in any case, organisms of this type do not cause any major problems in the yoghurt industry. Thus, the use of cloth filters is more than adequate for raw milk. In some instances, an in-line metal sieve has to

be installed when dried milk products are used to fortify the total solids in the milk; the metal sieves serve to separate any scorched or undissolved milk powder particles.

2.2.3 Milk reception and storage

Milk collection from farms in developing and industrialised countries is carried out in bulk, using a road tanker and, in some instances, rail tanker, or in churns; the facilities available for milk reception at a typical dairy are discussed in Chapter 3. However, the current practice of milk handling in dairies involves (a) ensuring that the temperature is about 5°C, (b) perhaps subjecting the milk to various treatments before storage such as thermising at about 65–67°C and cooling to <5°C, inoculating the milk with lactic acid bacteria or other microfloras to control the growth of psychrotrophic bacteria (Fetlinski *et al.*, 1982; Bianchi-Salvadori and Lavezzari, 1984), and/or (c) addition of formate or flushing with CO₂ (Singh and Shankar, 1984; Roberts and Torrey, 1988; Ruas-Madiedo *et al.*, 1996; Espie and Madden, 1997). Muir (1996) reviewed these methods of milk preservation and their effect on the quality of fresh dairy products. However, the use of CO₂ can cause the deposition of milk solids in a plate heat exchanger and degassing is recommended before heat treatment (Calvo and de Rafael, 1995). Milk containing somatic cells >250 000 ml⁻¹ can affect the organoleptic properties of yoghurt (Rogers and Mitchell, 1994), and whilst preculturing the milk with proteolytic enzymes (from psychrotrophic bacteria or plasmin) or prolonged storage of milk for up to 6 days at about 7°C stimulates the growth of the starter culture, the yoghurt has substantially different physical properties (Reinheimer *et al.*, 1990; Gassen and Frank, 1991; Srinivas *et al.*, 1997; Prabba and Shankar, 1997).

In warm countries milk tends to deteriorate faster due to methods of production and handling. A handbook has been published by the International Dairy Federation (IDF, 1990) that addresses this topic in detail and the measures that are used to minimise the bacterial spoilage of milk. Furthermore, the lactoperoxidase (LP) system delays gel formation in cow's milk by 1.5 hours and affects the flavour of the yoghurt; the body and texture characteristics are not affected (Mehanna and Hefnawy, 1988; Kumar and Mathur, 1989; Abdou *et al.*, 1994; Nichol *et al.*, 1995; Nakada *et al.*, 1996).

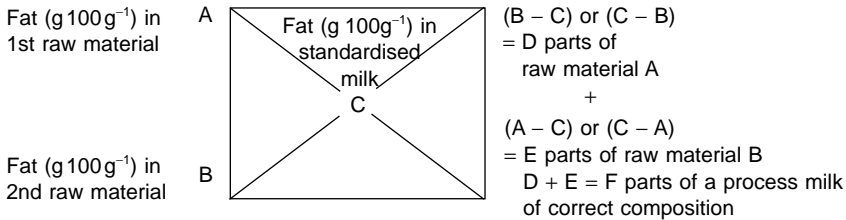
2.3 Standardisation of fat content in milk

The fat content (g 100 g⁻¹) of yoghurt manufactured in different parts of the world can vary from as low as 0.1 to as high as 10 and in order to meet existing or proposed compositional standards for yoghurt, it is necessary to standardise the milk. For example, a typical average butterfat content in milk ranges from 3.7 to 4.2 g 100 g⁻¹ (Fig. 2.3), but the fat content of commercial yoghurt averages around 1.5 g 100 g⁻¹ (medium fat yoghurt) or 0.5 g 100 g⁻¹ (low fat yoghurt). The methods employed for standardisation are as follows:

- removal of part of the fat content from milk
- mixing full cream milk with skimmed milk
- addition of cream to full fat milk or skimmed milk

- a process which may combine some of the methods mentioned above, that is, the use of standardising centrifuges.

The components required to achieve a standard milk, using one of the above methods, can be easily calculated using the Pearsons Square method.



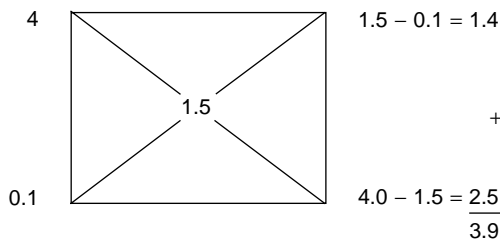
Alternatively, to calculate the amount of each type of raw material required, for example, per batch of a 1000l of standardised milk:

$$A = \frac{(B - C) \text{ or } (C - B) \times 1000}{F}$$

$$B = \frac{(A - C) \text{ or } (C - A) \times 1000}{F}$$

1st example

How many litres of full cream milk (4g fat 100g⁻¹) and skimmed milk (0.1g fat 100g⁻¹) are required to produce 1000l of yoghurt milk at 1.5g fat 100g⁻¹?



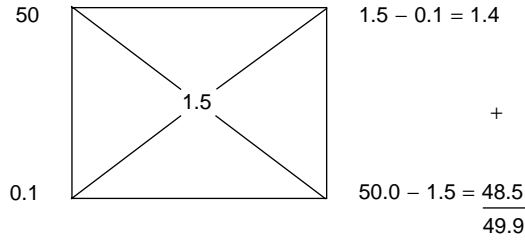
The amount of full cream milk required = $\frac{1.4 \times 1000}{3.9} = 3591$

The amount of skimmed milk required = $\frac{2.5 \times 1000}{3.9} = 6411$

Total 10001

2nd example

How many litres of cream (50g 100g⁻¹) and skimmed milk (0.1g fat 100g⁻¹) are required to produce 1000l of yoghurt milk at 1.5g fat 100g⁻¹?



The amount of cream required $= \frac{1.4 \times 1000}{49.9} = 28.11$

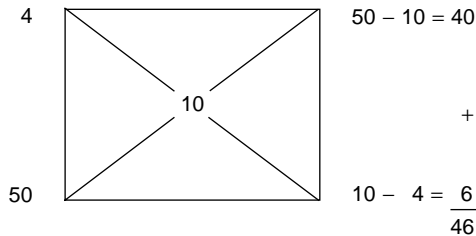
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The amount of skimmed milk required $= \frac{48.5 \times 1000}{49.9} = 971.91$

Total 1000.01

3rd example

How many litres of cream (50g 100g⁻¹) and full cream milk (4g fat 100g⁻¹) are required to produce 1000l of yoghurt milk at 10g fat 100g⁻¹?



The amount of full cream required $= \frac{40 \times 1000}{46} = 869.61$

The amount of cream required $= \frac{6 \times 1000}{49.9} = 130.41$

Total 1000.01

2.4 Standardisation of the solids-not-fat content in milk

The percentage of solids-not-fat (SNF) (mainly the lactose, protein and mineral matter) in milk for the manufacture of yoghurt is governed either directly by legal standards of the country concerned, or indirectly by the manufacturer seeking to produce an end product with certain physical properties and flavour. In the case of existing legal standards, the required solids-not-fat content in yoghurt ranges from 8.2 to 8.6g 100g⁻¹ (see Chapter 10), and this minimum percentage seeks merely to protect the consumer; that is, the SNF level is roughly comparable to the level present in liquid milk. From the manufacturer's point of view, the physical

properties of yoghurt, for example, viscosity/consistency of the coagulum, are of great importance and, in general, the higher the level of solids in the yoghurt mix the greater the viscosity/consistency of the end product. The relationship between the level of solids in the milk and the consistency of yoghurt was studied by Tamime (1977), and he observed that this property was greatly improved as the milk solids increased from 12 to 20 g 100 g⁻¹. Figure 2.4 shows this improvement in consistency as measured by the penetrometer. It must be emphasised that the greater the depth of penetration, the softer the coagulum and vice versa. However, the change in consistency between 16% and 20% tends to be less pronounced and hence there may be little value, in terms of product quality, in using a solids level above 16 g 100 g⁻¹.

Since the 1970s, there have been many publications on the technology of yoghurt and other fermented milk products (Humphreys and Plunket, 1969; Robinson and Tamime, 1975, 1986, 1990, 1993; Rasic and Kurmann, 1978; Tamime and Deeth, 1980; Olano and Ramos, 1982; Bottazzi, 1983; Kilara and Treki, 1984; Merilainen, 1987; Shukla *et al.*, 1987; Roginski, 1988; Tamime and Robinson, 1988; Morgensen, 1988; Chandan, 1989; Ferguson, 1989; Kroger *et al.*, 1989, 1992; Schmidt, 1992; Chandan and Shahani, 1993, 1995; Rossi, 1994; Varnam and Sutherland, 1994; Sarkar, 1995; Tamime and Marshall, 1997; Oberman and Libudzisz, 1998). However, in a series of articles, Vedamuthu (1991a–h, 1992a, b) has reviewed the topic extensively, while Mann (1984, 1985, 1987, 1990a, b, 1992a, b, 1994a, b) regularly publishes a “Digest” of international dairy publications on yoghurt. Furthermore, the International Dairy Federation periodically publishes monographs updating the technological and scientific aspects of fermented milks (IDF, 1984, 1988a, 1992a).

The level of solids in milk (including the fat content) for the manufacture of yoghurt ranges from as low as 9 g 100 g⁻¹ in low fat yoghurt to as high as 30 g 100 g⁻¹ in other types of yoghurt. The best yoghurt is probably made from milk containing 15–16 g 100 g⁻¹ total solids (Tamime *et al.*, 1987) and the composition of

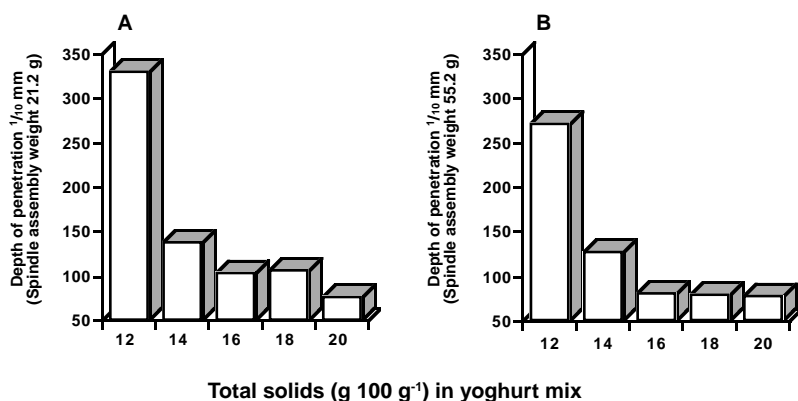


Fig. 2.4 Consistency measurement of yoghurt (12–20 g total solids 100 g⁻¹) directly at the end of the incubation period at 42°C (A) and after overnight storage in a refrigerator at 5–7°C (B)

Note: A standard penetrometer was employed.

After Tamime (1997).

Table 2.3 Effect of total solids in the mix in relation to natural acidity (NA), titratable acidity (TA) and developed acidity (DA) after incubation at 42°C

Total solids (g 100 g ⁻¹) in yoghurt milk	Time of incubation (hours)	% Lactic acid		
		NA	TA	DA
12	3.5	0.15	0.80	0.65
14	3.5	0.19	0.84	0.65
16	3.0	0.21	0.83	0.62
18	2.5	0.24	0.88	0.64
20	3.0	0.29	0.93	0.64

Full cream spray dried milk powder was reconstituted to different levels of total solids in the mix.

Starter culture was CH-1 obtained from Chr. Hansen's Laboratorium A/S, Copenhagen, Denmark.

Adapted from Tamime (1977).

most commercial yoghurts falls within the range of 14–15 g 100 g⁻¹. Although 30 g 100 g⁻¹ total solids has been suggested for the production of “super” yoghurt, the end product could well resemble “concentrated” yoghurt in its consistency rather than normal yoghurt (see Chapter 5). Furthermore, if the total solids level in the yoghurt mix is in excess of 25 g 100 g⁻¹, it can adversely affect the availability of moisture to certain strains of starter culture and this in turn can hinder their activity (Pulay and Krasz, 1974; Patel and Chakraborty, 1985).

As a result of increasing the level of SNF in the mix, the titratable acidity of the milk is raised due to the buffering action of the additional proteins, phosphates, citrates, lactates and other miscellaneous milk constituents (Walstra and Jenness, 1984) and this function can lead to a reduced gel formation time (Table 2.3). A similar view is held by Davis (1973), who reported that doubling the SNF content in milk resulted in a doubling of its titratable acidity. However, different levels of SNF in milk influenced the generation times and cell counts of the yoghurt starter culture; optimum conditions were 12 g and 14 g SNF 100 g⁻¹ for *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, respectively (Al-Dabbagh and Allan, 1989).

The fortification of the total solids in the yoghurt mix can be achieved by a number of different methods, such as those described in the following sections.

2.4.1 Traditional process

The application of heat to milk has long been practised traditionally, that is, boiling to reduce the volume of the milk to two-thirds of its original value. Although the objective was to increase the concentration of total solids in the milk, the application of heat caused many physicochemical changes (refer to Section 2.9 on heat treatment). The degree of concentration achieved by the boiling process is rarely calculated with any accuracy, but if, for example, the total solids level in the milk is 13 g 100 g⁻¹, the result of boiling the milk to reduce its volume to two-thirds will be to raise the total solids content to around 19–20 g 100 g⁻¹. This method of fortification is still used in rural communities where the scale of yoghurt manufacture is very small.

2.4.2 Addition of milk powder

Milk powder (full cream or skimmed) is widely used in the industry to fortify liquid milk for the manufacture of a thick smooth yoghurt (Bøjgaard, 1987). Since the majority of the commercial yoghurt produced in the United Kingdom is of the low fat type, it is probable that skimmed milk powder (SMP) is the more popular ingredient. The rate of addition to the yoghurt mix may range from as little as 1% to as high as 6%, but the recommended level is 3–4%, since the addition of higher levels of milk powder may lead to a powdery taste in the yoghurt.

Good quality yoghurt has been produced by fortification of the yoghurt mix with (a) 2% SMP (Wolfschoon-Pomba *et al.*, 1984; Resubal *et al.*, 1987; Mehanna, 1988; Mehanna and Hefnawy, 1990), (b) mixing raw milk with recombined milk at a ratio of 1:1 (Kurwijila *et al.*, 1983; Caric *et al.*, 1986; Balasubramanyam *et al.*, 1988), (c) replacing half the water required for recombination of SMP with sweet whey (El-Safty and El-Zayat, 1984) or using only Cheddar cheese whey (Krishna *et al.*, 1984), and (d) addition of high protein SMP to increase the level of protein to 5.2 g 100 g⁻¹ (Mistry and Hassan, 1992).

In some developing countries, yoghurt is manufactured totally using SMP and anhydrous milk fat (AMF), about 99.9 g fat 100 g⁻¹, and the normal practice is to rehydrate the powder to about 12 g 100 g⁻¹ SNF. The use of SMP during the manufacture of fermented milks is preferable to whole milk powder because of the problem(s) associated with oxidised flavour in the latter product (Harper, 1985). The latest approach in SMP production is the use of protein adjustment in order to overcome the seasonal variation in the protein content in milk, and to improve functional characteristics and storage stability (Kieseker and Healey, 1996). However, in some countries, for example Denmark and Italy, the fortification of the yoghurt milk with powder(s) is not permitted, and hence other methods are employed to increase the solids level.

High protein milk powders (whole or skimmed) are available in some markets, and these are produced by ultrafiltration followed by diafiltration in order to reduce the lactose content before drying (see Table 2.4) (Bjerre, 1990; Mistry and Hassan,

Table 2.4 Comparison of gross compositional quality (g 100 g⁻¹) of different powders used for the manufacture of yoghurt

Powder	Protein	Fat	Lactose
Whole milk			
Commercial	26.3	26.3	39.4
Retentate	41.7	41.7	9.3
Skimmed milk			
Commercial	36.1	0.6	52.9
Retentate ^a	62.8–80.5	0.9–1.5	5.5–23.9
Whey			
Commercial	12.2	1.3	78.0
Demineralised	14.5	1.0	80.5
Concentrate ^a	35.0–73.2	0.2	12.0–55.0
Buttermilk	34.1	5.0	51.0
Caseinate	87.3	0.2	–

^a Range of different powders.

Adapted from Tamime and Marshall (1997).

1991a, b; Mistry *et al.*, 1992; Aguilar and Ziegler, 1994a, b). They have been used to produce firm yoghurts (El-Samragy *et al.*, 1993a, b; Thomopoulos *et al.*, 1993; Panfil-Kuncewicz *et al.*, 1994; Getler *et al.*, 1997), but are more expensive than SMP.

Since SMP is widely used for recombination during the manufacture of yoghurt, the specifications of the powder are important and can influence the quality of the product. The current specifications for powders published by the American Dairy Products Institute (ADPI, 1990) are universally recognised; previously the organisation was known as the American Dry Milk Institute (ADMI). In general, powders should be free from any inhibitory agents and be of good microbiological quality and physical standards. Critical reviews of dairy powder specifications, including an update of standards, have been reported by Sjollem (1988) and Kjaergaard-Jensen (1990). Some specific requirements of SMP used for recombination have been reported by Wilcek (1990) and include the following:

- whey protein nitrogen index, 4.5–5.9;
- cystein number, 38–48;
- thiol number, 7.5–9.4;
- heat number, 80–83.

These specifications classify the powder(s) as medium heat which is ideal for the production of fermented milks. Furthermore, the effect of thermal processing during whole milk powder production and storage temperature can affect its quality (Caric and Kalab, 1987; McKenna, 1997) and that of any yoghurt into which it is made (McKenna and Anema, 1993). As a consequence, New Zealand Milk Products have launched ALACO, a range of special powders that are texture improvers for yoghurt (Russel, 1994; Anon., 1994a; Harnett and Muller, 1995). A similar powder has been developed by DMV International in Holland called Excellion containing about 51–85 g protein 100 g⁻¹ which is suitable as a SMP/stabiliser replacement (Maas, 1997); however, its functional characteristics are to improve the viscosity, texture and mouthfeel of yoghurt, and reduce syneresis.

The quality of yoghurt was studied using different commercial types of SMP (Klupsch, 1987, 1989; Blondeau and Goursaud, 1992) and the characteristics of the product (i.e. flavour, texture and acidity) differed considerably; some powders were suitable for set rather than stirred-type yoghurts. Recently, Chung *et al.* (1997a, b) reported that the use of old SMP affected the quality of yoghurt, so confirming that powder specifications can affect the quality of the manufactured yoghurt.

2.4.3 Addition of buttermilk powder

Buttermilk powder (BMP) is a by-product of sweet cream butter manufacture, but an acid type can also be obtained from the churning of cultured cream. This low fat powder is of value to the food and dairy industry because, due to the presence of high levels of phospholipids, it has considerable emulsifying properties and its chemical composition is similar to SMP. A method of manufacturing yoghurt from recombined dairy ingredients has been reported by Gilles and Lawrence (1979, 1982); the suggested formula is: 25 kg AMF, 125 kg SMP, 10 kg buttermilk powder and 840 kg water.

Buttermilk powder, used up to 50% as a replacement for SMP in the manufacture of low fat yoghurt, was acceptable and similar to the control product (Vijayalakshmi *et al.*, 1994). Fresh buttermilk fortified with SMP has been used

successfully to produce good quality yoghurt (El-Batawy *et al.*, 1987; Vodickova *et al.*, 1987; Mansour *et al.*, 1994/95), but the use of fresh buttermilk concentrated by ultrafiltration (UF) or nanofiltration (NO) in low fat yoghurt production affected the consistency, flavour and aroma but not product stability (Reierstad, 1993).

2.4.4 Addition of whey powder and/or whey protein concentrates

This product originates in the cheese industry, and its utilisation in the food and the dairy industry was reviewed by Zadow (1983, 1994a, b), Alais and Blanc (1975), Smith (1976), Robinson and Tamime (1978), IDF (1988b) and Sienkiewicz and Riedel (1990). There are many different types of whey powder (WP) (e.g. whey protein concentrates (WPC), isolate (WPI) or hydrolysate (WPH), denatured whey protein, whey protein fraction and non-protein nitrogen product) available on the market and the characteristics of each are related to the processing technique applied before the drying stages, for example, demineralisation, lactose removal, whey protein concentrate or straightforward drying. The production and utilisation of concentrated whey proteins have been reported by Howel *et al.* (1990), Morr and Foegeding (1990), Dybing and Smith (1991), Wilmsen (1991, 1992), Harper (1992), IDF (1992b), Caric (1994), Barbut (1995), Blenford (1996) and Urbiene and Leskauskaitė (1996). According to Jelen and Horbal (1974), Hartman (1975), Nielsen (1976) and Spurgeon (1976), the recommended level of addition of whey powder to the yoghurt mix is around 1–2%, since higher levels can impart an undesirable whey flavour. However, a process for the preparation of a yoghurt flavour is based on fermenting cheese whey followed by drying (van der Schaft, 1991) and the addition of such product to yoghurt improves its flavour and sweetens it.

Since the 1970s, there have been great developments in whey technology to produce various products of specific functional characteristics for yoghurt making. The heat stability of whey protein during the manufacture of yoghurt was reported by Buchheim *et al.* (1986), Jelen *et al.* (1987), Patocka *et al.* (1993) and Hollar *et al.* (1995). However, whey protein powder was used to fortify the yoghurt mix at levels ranging between 0.6% and 4% (Guirguis *et al.*, 1984, 1987; Mehanna and Gonc, 1988; Rockell, 1989; Timmermans, 1993; Venkateshaiah and Jayaprakasha, 1995; Morris *et al.*, 1995; Venkateshaiah *et al.*, 1996; Kailasapathy and Supriadi, 1996; Kailasapathy *et al.*, 1996a, b) and the results showed (a) that more acetaldehyde was produced, (b) increased viscosity and reduced syneresis, (c) improved sensory attributes and (d) enhanced buffering capacity at low pH. Good yoghurt could be produced from recombining SMP and sweet WP in a ratio of 75:25 (solids content about 12 g 100 g⁻¹), but a higher ratio of 50:50 was recommended for yoghurt made with 75% lactose hydrolysis; the latter product contained higher levels of soluble nitrogen due to:

- the addition of WP;
- the carry-over of yeast proteolytic activity in the β -D-galactosidase preparation, and
- the activity of the starter culture (Shah *et al.*, 1993).

Replacement of SMP by whey–caseinate blends at 50% reduced the cost of manufacture and the yoghurt was acceptable, but the application of lactose hydrolysis during the manufacture of yoghurt has raised the cost slightly (Whalen *et al.*, 1988). Furthermore, different processes for the manufacture of yoghurt and related products using whey protein powder(s) in the mix have been patented by

Koenraads and de Boer (1986), Tomita *et al.* (1992) and Hirano *et al.* (1994) (see also Hoffmann *et al.*, 1998).

WPC (i.e. about 14 g total solids (TS) 100 g⁻¹) have been used to fortify the yoghurt mix at a level up to 30% without affecting the quality of the product (Broome *et al.*, 1982; Greig and van Kan, 1984; Gruev and Flejtás, 1985; Tratnik and Krsev, 1985, 1988; Hofi *et al.*, 1994/95; Maric *et al.*, 1997). Greig and Harris (1983) observed a “cheesy” odour and a reduction in the viscosity of the yoghurt ($P < 0.01$) when the substitution of liquid milk was 40% with WPC, and the best results were obtained with 10% substitution, while Abou-Dawood *et al.* (1984) recommended the use of WPC to increase the SNF by 1 g 100 g⁻¹.

In Egypt, salted whey from Domiati cheese was demineralised twice (about 12.51 g TS 100 g by UF) to reduce the salt content, and the WPC was used successfully to replace 40% of the milk in the yoghurt mix (Abd-Rabo *et al.*, 1988). Alternatively, the salted whey could be UF and diafiltered using sweet whey rather than water, and finally the WPC was diluted with sweet whey to adjust the protein level to 3.5 g 100 g⁻¹ (Abd El-Salam *et al.*, 1991). Such WPC was added to buffalo's milk up to 20% and the manufactured yoghurt had a better texture, mouthfeel and reduced syneresis. Cottage cheese whey was concentrated by vacuum evaporator (VE) to 40 g TS 100 g⁻¹ by Baig and Prasad (1996) and part of this was acidified to pH 4.6 to produce a more acid WPC. Both whey concentrates were used separately to replace SMP in yoghurt making and the results were satisfactory, but it was observed that the incorporation of whey solids stimulated the growth of *S. thermophilus* and *Bifidobacterium bifidum*, whilst the counts of *L. delbrueckii* subsp. *bulgaricus* were reduced. Nevertheless, the firmness and syneresis of “cream” yoghurt (about 10 g fat 100 g⁻¹) made from milk and concentrated Camembert cheese whey were significantly influenced by the ratio of casein to whey and an optimum range recommended was 1.2–2.2 (Kulkarni *et al.*, 1990a, b; Plock and Kessler, 1992).

Recently, de Boer and Koenraads (1992) have reviewed the application of liquid WPC for partial replacement of skimmed milk during the manufacture of yoghurt in terms of: (i) legislation in most European countries is far from being uniform; however, based on the legal specifications in the Netherlands, the permitted and maximum replacement of SNF in milk with WPC was 10%, 20% and 30% for drinking yoghurt, plain stirred yoghurt and fruit stirred yoghurt, respectively, (ii) the microbial activity of the yoghurt starter during the fermentation stage was slightly enhanced, possibly due to shorter lag phase of *L. delbrueckii* subsp. *bulgaricus*; however, a stimulating effect was observed with *Lactobacillus acidophilus* (Marshall *et al.*, 1982), but contradictory results with yoghurt starter cultures have been reported in the literature which could be due to strain variation, and (iii) the rheological and sensory properties of yoghurt were, in some instances, improved and in other cases the flavour was affected. This could be attributed to: (a) the level of fortification of WPC used, and (b) the processing conditions applied during the preparation of the yoghurt mix. However, variations in the properties of the WPC during its preparation should not be overlooked.

2.4.5 Addition of casein powder

Different types of casein powder (e.g. acid or rennet casein, Na-, K-, Ca- or NH₃-caseinate and casein hydrolysate) are manufactured from skimmed milk and their

properties vary according to the technique used to precipitate the original casein, for example, acid casein (hydrochloric, lactic or sulphuric acid precipitation), coprecipitated casein and rennet casein. Casein powder, as the name indicates, consists mainly of casein and its addition to the yoghurt mix increases both the level of protein in the product and its viscosity (Sen, 1985; Hendrickx, 1996); the level of addition, compared with SMP, is comparatively low (see Fig. 2.5).

It is evident that different powders could be used to fortify the protein content in the yoghurt mix (see Table 2.4) and depending on the type of powder used, the physical and sensory properties could be influenced and/or modified. Caric (1994) has reviewed the different techniques used for the production of powders including whey protein concentrates. The functional properties of WPC have been reported by Kinsella (1986) and Kjaergaard-Jensen *et al.* (1987), whilst Robinson and Tamime (1986) have reviewed the role of protein(s) in yoghurt making.

The quality of yoghurt made with different dried ingredients has been investigated by many researchers in different laboratories around the world. Some examples of ingredients used to produce good quality yoghurt include: (a) mixing Ca-caseinate and whey powder in a ratio of 1:1 (Conc and Uysal, 1994), (b) the addition of Na-caseinate gave a firm yoghurt with little syneresis, whilst the differences between the addition of SMP and dried milk proteins were marginal; however, correlations between the sensory and rheological properties of the yoghurts made with different ingredients were influenced by the type of starter cultures used (Rohm and Kneifel, 1993), and (c) the susceptibility to syneresis of yoghurts made with different dried ingredients decreased in the following order: WPC 35 > Na-caseinate > WPC 45, 60 or 75 > SMP > BMP, whilst the viscosities, after a 25 min shear at a rate of 116.2 s^{-1} , decreased in the order: Na-caseinate > BMP, SMP, WPC 75, 60 or 45 > gelatin > WPC 35 (Guinee *et al.*, 1994, 1995; see also Rhom, 1993a; Rhom and Schmid, 1993) (note: the numbers refer to percentages of protein).

In some instances protein hydrolyates have been recommended for use during the manufacture of yoghurt. Casein hydrolysates containing peptide lengths of about 1.5 stimulated the growth of *S. thermophilus* due to the increase in free amino

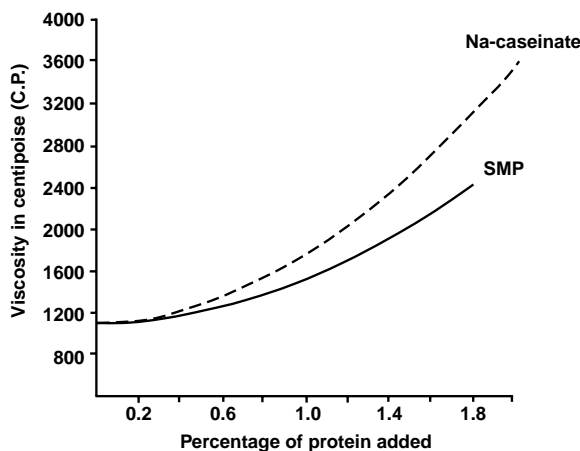


Fig. 2.5 Effect of an increase in protein content on viscosity of yoghurt

After van Gennip (1973, 1980, 1981a, b).

acids (Nakamura *et al.*, 1991). However, the addition of up to 1% casein digest increased the viscosity by 16–87% and reduced syneresis by 26.5–30% in yoghurts made using strains of *S. thermophilus* (i.e. high and low viscosity) isolated from commercial yoghurts (Kim and Hwang, 1996). Alternatively, yeast autolysate and hydrolysed protein can be added at a rate of 0.5–0.3 g 100 g⁻¹ to cultured milk products to control lipolysis and enhance the flavour (Akatsuka, 1984), whilst hydrolysate obtained from the muscle of mackerels (*Scomber japonicus*) has stimulated the growth of *S. thermophilus* in milk fortified by 0.2–1.0 g 100 g⁻¹, but not *L. delbrueckii* subsp. *bulgaricus* (Lee and Kim, 1986). Soy bean protein isolates and/or yeast extracts when added to milk have been found to stimulate the growth of *S. thermophilus* and different species of bifidobacteria (Yajima *et al.*, 1992). However, the addition of such hydrolysates to fermented milks may be governed by statutory regulation.

2.4.6 Concentration by vacuum evaporation (VE)

This method of concentrating the total solids in the yoghurt mix is widely used in the industry. For an illustration of a typical plant refer to Chapter 3. The basic requirement is a single effect plate evaporator which can be easily incorporated into a yoghurt processing line. The evaporation and/or concentration process is carried out on the milk before the final heat treatment. In practice, the yoghurt milk must first be standardised, for example, the fat content, since the evaporation concentrates all the milk constituents with the exception of minor losses of volatile compounds in the condensate. The amount of water removed from the milk ranges from 10 to 25%, equivalent to an increase in the TS of 2–4 g 100 g⁻¹. However, Baltadzhieva *et al.* (1987) recommended VE of whole milk to 16–18 g TS 100 g⁻¹ for the production of good quality yoghurt. Some other advantages claimed for the evaporation process are first, the removal of water from the milk takes place under vacuum which, in turn, aids the removal of entrapped air and hence improves the stability of the coagulum and reduces syneresis during storage (Gradhage and Thurell, 1978). Second, during the manufacture of goat's milk yoghurt, the evaporation process improves the consistency of and reduces the “goaty” flavour of the end product (Hadland and Hoffmann, 1974).

Incidentally, under commercial practice the yoghurt milk could be fortified and/or standardised using concentrated milk (whole or skimmed) or WPC from factories producing such concentrates before the drying stage. Typical gross compositions of concentrated milk products is shown in Table 2.5.

2.4.7 Concentration by membrane filtration

Membrane filtration is a process which was developed to concentrate and/or separate solids from an aqueous mixture. The usual membrane processes are reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF). The applications of RO and UF in the dairy industry have been reviewed by Glover *et al.* (1978), Hedrick (1983/84), Glover (1985), IDF (1979, 1992b, 1996), Kosikowski (1986), McGregor (1986), Cheryan (1986), Maubois (1989), Mohr *et al.* (1989), Rao *et al.* (1989), Degremont (1991a, b), Renner and Abd El-Salam (1991), Kessler *et al.* (1991), Grandison and Glover (1994), Caric (1994) and Bird (1996). The major functional differences between RO, NO, UF and MF are as follows:

Table 2.5 Comparison of gross compositional quality (g 100 g⁻¹) of concentrated milk products

Concentrate	Protein	Fat	Lactose
Evaporated milk			
Whole	6.8	7.6	10.0
Skimmed	7.5	0.2	11.3
Condensed			
Buttermilk	9.9	1.5	12.0
Whey	7.0	2.4	38.5
Retentate			
Whey ^a	12.2	NR	3.2

^a Ultrafiltered; the volume of concentration factor is ×20.
NR: not reported.
Data compiled from Bassette and Acosta (1988) and Caric (1994).

- The RO process separates very low molecular weight solutes, i.e. about 100, and only water molecules are allowed to pass through the membrane. Thus, the membranes are basically impermeable (or slightly permeable) to organic compounds or inorganic ions and consequently the osmotic pressure becomes an important feature in the process. The RO system is operated at high pressures, i.e. 1–6 megapascals (MPa).
- The NO process is sometimes known as ultraosmosis. This system of filtration separates selectively low molecular weight solutes from aqueous solutions. The membranes are more permeable than RO, but less permeable than UF membranes. The NO system normally operates at pressures of 2–3 MPa.
- The UF process merely sieves or filters the milk and the membranes can only retain high molecular weight fractions, i.e. >2000. The operating pressures are, therefore, much lower than with the RO process, e.g. 0.1–1 MPa.
- The MF process operates at a very low pressure (about 0.01–0.05 MPa) and it is used to separate suspended particles up to 10µm from an aqueous solution.

The material that passes through the membrane is referred to as the permeate, and the part of the feedstock which is retained by the membrane and contains the solute(s) or constituent(s) to be concentrated is referred to as the retentate. Thus in principle, the permeate will be deficient in the solute(s) that are concentrated. Table 2.6 illustrates the permeability of solutes in milk (whole or skimmed) or whey using different membranes. The major difference between the permeates is that, while the RO permeate consists only of water, the UF permeate contains lactose, non-protein nitrogen, organic acids, ash and water-soluble vitamins besides water. A comparison of the chemical compositions of whole milk, skimmed milk and whey concentrated by RO and UF (and their permeates) is illustrated in Table 2.7.

Membrane filtration techniques are utilised in the dairy industry for specific processes (Bylund, 1995; Bird, 1996), and some examples are:

- RO is used for concentrating whey, UF permeate and, to a lesser degree, yoghurt milk.
- NF is applied for partial demineralisation/desalination of whey, UF permeate or retentate.

Table 2.6 Permeability of mik and/or whey components through different membrane systems

Milk/whey components	Type of membrane system			
	RO	NO	UF	MF
Water	✓	✓	✓	✓
Minerals	R	✓	✓	✓
Lactose, AA and NPN	R	R	✓	✓
Proteins	R	R	R	✓
Fat and bacteria	R	R	R	R

✓: passes through the membrane into the permeate; R: rejected by the membrane and retained in the retentate. AA: amino acids. NPN: non-protein nitrogen.

Adapted from Bird (1996).

Table 2.7 Chemical composition (g100g⁻¹) of the permeate and retentate of milk (whole and skimmed) and whey after concentration by UF or RO

Process	Product	Concentration factor	Total solids	Fat	Protein	NPN	Lactose
UF	Whole milk	×3	12.9	3.9	3.1	0.18	4.7
	Permeate		6.1	–	0.06	0.19	5.1
	Retentate		28.6	12.6	9.8	0.18	4.1
	Skimmed milk	×3	8.5	–	3.1	0.17	5.1
	Permeate		5.7	–	0.06	0.17	5.3
	Retentate		15.5	–	9.3	0.20	4.7
	Whey	×20	6.0	–	0.68	0.29	4.0
	Permeate		6.2	–	0.13	0.55	4.5
	Retentate		20.1	–	12.12	2.11	3.2
RO	Whole milk	×2	11.7	3.2	3.1	–	4.3
	Permeate		0.08	–	–	–	–
	Retentate ^a		22.6	6.4	6.1	–	8.6
	Skimmed milk	×2.2	8.8	–	3.1	–	4.7
	Permeate		0.33	–	–	–	–
	Retentate ^a		19.0	–	6.9	–	10.3
	Whey	×2.7	6.8	–	0.8	–	4.4
	Permeate		0.11	–	–	–	–
	Retentate ^a		18.2	–	2.2	–	11.9

NPN: non-protein nitrogen. Dashes (–) represent data not reported or in the case of RO filtration valve is nil.

^a Data calculated from the membrane retention percentage reported.

Dated compiled from Glover (1971, 1985).

- UF process concentrates the fat and proteins in milk (see Fig. 2.6) for the standardisation of cheese milk or fortification of the yoghurt milk. It is also used to concentrate whey for the production of WPC.
- MF is basically used to reduce the number of micro-organisms in skimmed milk, whey and brine, and also to de-fat the whey used for the production of WPC or WPI.

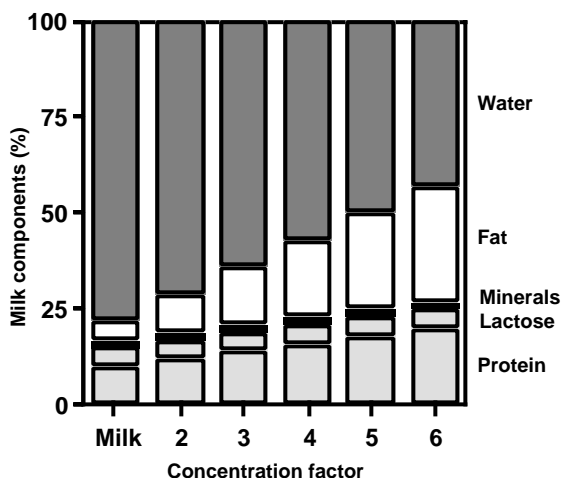


Fig. 2.6 An illustration showing the effect of concentration of components in whole milk UF retentate

Note: fat and protein contents increase in relation to the concentration factor whilst the lactose and minerals remain constant; the latter constituent increases marginally at ≥ 5 fold.

After Puhan (1990a, b).

The industrial-scale production of yoghurt from milk concentrated by RO or UF has been reported by Jepsen (1977, 1979) and according to the data compiled by Tamime and Deeth (1980) and Ferguson (1989), the qualities of yoghurt produced from RO and UF concentrated milks are as follows. First, whole milk concentrated by UF to $18\text{--}20\text{g TS } 100\text{g}^{-1}$ produced a smooth, creamy yoghurt with a typical acid flavour; homogenisation was not required during subsequent treatment of the milk. Second, a process similar to the one mentioned above, but with the lactose content adjusted to $2\text{g } 100\text{g}^{-1}$, resulted in a yoghurt rated as superior to ordinary brands. Third, skimmed milk concentrated by UF to $13\text{g TS } 100\text{g}^{-1}$ was also suitable for yoghurt making. Fourth, the manufacture of yoghurt from skimmed milk concentrated by RO to $15\text{g } 100\text{g}^{-1}$ total solids resulted in a yoghurt of similar quality (viscosity, acid and flavour) to yoghurt produced from skimmed milk fortified to $15\text{g } 100\text{g}^{-1}$ total solids with SMP.

It is safe to assume that the application of RO in yoghurt making is very limited in the industry when compared with UF. Nevertheless, Dixon (1985) made yoghurt from RO retentate (i.e. the volume of milk was halved after concentration) which had a higher apparent viscosity and was less susceptible to syneresis than yoghurt where the milk was fortified with SMP. Some studies on yoghurt made from UF retentate suggest the following aspects and/or recommendations. First, Atamer *et al.* (1990) suggested that the total solids content should be $13.23\text{g } 100\text{g}^{-1}$, but no data were given regarding the fat level; however, recombined SMP concentrated by UF to $12\text{g } 100\text{g}^{-1}$ solids plus AMF and later made into yoghurt was highly rated by a taste panel (Mehanna *et al.*, 1988). Second, yoghurt made from UF retentate had a total free amino acids to protein ratio of 0.0375 and no significant difference was observed in particle size in low molecular weight peptides of yoghurt (Nakazawa *et al.*, 1991). However, concentrating milk more than two-fold resulted in a product which was too firm; curd tension was correlated with the degree of concentration

(Tomita *et al.*, 1987). Third, low lactose (about $0.75\text{ g }100\text{ g}^{-1}$) and low sodium yoghurt was produced by combining UF (about 20 kDa) with addition of β -galactosidase to the retentate, and pectin, calcium phosphate and apple concentrate were added to the milk to compensate for calcium losses during filtration (Rasic *et al.*, 1992). Fourth, the relative viscosity of skimmed milk UF retentate at pH 6.0 was influenced by protein content and $1\text{ g NaCl }100\text{ g}^{-1}$ (Abd El-Salam *et al.*, 1987). Fifth, skimmed milks concentrated by UF (about 10 kDa) and RO were made into yoghurt; the former had a protein to lactose ratio of 1.2 and produced good yoghurt when the protein content was increased by 35% (Brazuelo *et al.*, 1995).

It is evident that the activity of starter cultures in UF retentate is greater when an increase in conductance is observed, and the change in pH is decreased despite an increase in lactic acid content (Lanzanova *et al.*, 1993). Such microbial behaviour is attributed to the buffering capacity of the UF retentate (Mistry and Kosikowski, 1985a, b, 1986a–c; see also Alvarez *et al.*, 1998).

2.4.8 Addition of non-milk proteins

In countries where there is a shortage of milk production for human consumption, proteins that originate from plant, animals and other sources have been used in research laboratories to fortify the milk during the manufacture of yoghurt. Examples of using non-milk protein may including the following:

- Soy-milk and its protein derivates have been used extensively in food formulations, and soy-based yoghurt products are reviewed in detail in Chapter 5.
- Sweet potato (SP), milk, sucrose and gelatin mixtures were used to make yoghurt containing high amounts of protein (about $19\text{ g }100\text{ g}^{-1}$). A good quality yoghurt was produced, but the product became darker in colour as the SP content was increased; overall, such yoghurts contained appreciable nutrients, for example, vitamin C $0.3\text{--}0.4\text{ mg }100\text{ g}^{-1}$, vitamin A 971–1252 retinol equivalent 100 g^{-1} and dietary fibre $2.5\text{ g }100\text{ g}^{-1}$. No reduction in starter activity was reported (Collins *et al.*, 1991a–c).
- Pulses or legumes, such as faba, cowpeas and mung beans, have been used in the preparation of yoghurt-like products. Faba bean yoghurt was highly rated in Egypt (Abou-Donia and Salam, 1981, 1982), but fermented milks made with cowpeas and mung beans were inferior to yoghurt, even though the sensory attributes were still within an acceptable range (Rao *et al.*, 1988; see also Ibrahim *et al.*, 1993).
- Egg white, soy-milk, gums, sugar, skimmed milk and vanilla extract were blended and processed into an acceptable and stable product (MaKenzie, 1983; Lin and Cunningham, 1984; see also Muller *et al.*, 1987).
- Sunflower protein was used for partial replacement of the milk proteins in yoghurt making; such proteins appeared not to have any gel-forming ability, but interacted with the caseins to form a soft gel yoghurt (Bilani *et al.*, 1989).
- Groundnut protein (flour or isolate) was blended with milk (whole with added SMP) to increase the total solids up to $23\text{ g }100\text{ g}^{-1}$; heating the milk at 80°C for 30 min gave a curd after fermentation with increased yield stress and the strength was influenced by the concentration of groundnut protein (Ramana and Ramanathan, 1992; see also Venkateshaiah *et al.*, 1994).

- Cottonseed proteins (i.e. different types) were used in yoghurt preparation and the most acceptable product, when compared with the control made from 100% milk powder, was obtained by mixing glandless cotton seed protein with whole milk powder in the ratios 1:1 and 1:3 (Abu-Foul *et al.*, 1992). Jiang *et al.* (1995) used a solution of fresh milk and low gossypol cottonseed protein at a ratio of 6:4, 1 g 100 g⁻¹ glucose and 0.1 g 100 g⁻¹ β -cyclodextrin to produce an acceptable yoghurt.
- Coconut milk fortified with SMP and the addition of 12 g 100 g⁻¹ sugar gave an acceptable product when compared with the control yoghurt (Sanchez and Rasco, 1984; Anon., 1985a, b; Davide, 1986).
- Dried egg white fortification of milk up to 3% enhanced acid development by *L. acidophilus*, *Lactobacillus paracasei* subsp. *paracasei* and *L. delbrueckii* subsp. *bulgaricus*, and the viscosity of the yoghurt was influenced by the amount of dried egg white used (Tae, 1997; Tae and Min, 1997).
- Miscellaneous protein additives such as soy-milk, oat flour and WP (Shirai *et al.*, 1992), wheat and milk proteins (Lorenzen, 1993), dried *Aloe vera* (Yongseo *et al.*, 1996; Lee and Yoon, 1997) and soy-milk and/or brown rice (Kisuk *et al.*, 1997) have been used to fortify milk to produce an acceptable yoghurt. The addition of mushroom extract (1 g 100 g⁻¹) (*Lentinula edodes*) to reconstituted SMP enhanced the rate of acid development by *L. delbrueckii* subsp. *bulgaricus*, but the coagulum had a coarse structure (Vargas and Ohashi, 1996, 1997).

There are many methods of fortification/standardisation of the fat and/or SNF content of the milk base. A comparison of the chemical composition of these potential ingredients is given in Tables 2.4, 2.5 and 2.7. The choice of any one particular method of fortification in a given situation is governed primarily by the following factors:

- cost and availability of the raw materials
- scale of production
- capital investment in the processing equipment

but it is important to note that the degree of supplementation of each of the different milk constituents does vary with the method used; the possible increases or decreases in the level of protein, lactose and fat contents in the yoghurt mix are dependent on the method of fortification/standardisation employed. However, other considerations may be equally relevant, and, for example, the addition of milk powder (whole or skimmed) beyond a certain level may result in a powdery flavour in the yoghurt and, due to the high level of lactose present in the mix, can also lead to excessive acid production during cold storage. Nevertheless, the viscosity/consistency of the coagulum is of primary importance during the manufacture of yoghurt and this feature is wholly dependent on the level of protein in the milk base; a relationship that is evident with respect to the variations in protein content of milk throughout the year (van Gennip, 1973, 1981a, b). Commercially, a high protein content in the yoghurt milk can be achieved by the addition of caseinate powder, concentrating the milk by the UF method or, to a lesser degree, by the addition of a high protein powder (whey or milk) and/or buttermilk powder (see Tables 2.4, 2.5 and 2.6).

Although, in broad terms, the overall level of protein in the mix affects the characteristics of the coagulum, the formation of the gel is entirely dependent on the

functional properties of the casein fraction (Rohm and Foissy, 1991). Thus, the lactic acid produced by the starter culture destabilises the casein micelles and at pH 4.6–4.7, in the presence of divalent ions (calcium and magnesium), the casein forms a three-dimensional network entrapping all the milk constituents including the aqueous phase. It is not surprising, therefore, that prior to the availability of high protein powders, the fortification of the milk base with casein or caseinate offered the following advantages:

- concentration of the milk, in order to increase the protein content, is not required;
- the natural flavour and texture of the yoghurt are maintained;
- it enhances the hydrophilic properties of the existing protein and so acts as a stabiliser;
- it improves the viscosity of yoghurt and decreases the problem of syneresis during cold storage;
- the recommended level of fortification, compared with skimmed milk powder, is in the proportion of 1 to 3, respectively. The efficacy of caseinate *vis-à-vis* skimmed milk powder in enhancing the consistency of yoghurt is shown in Fig. 2.5.

It is clearly feasible, therefore, to manufacture yoghurt from either concentrated or fortified milk. In an effort to isolate one particular method, Abrahamsen and Holmen (1980), Tamime *et al.* (1984), Becker and Puhan (1988, 1989) and Savello and Dargan (1995, 1997) compared the quality of yoghurt manufactured from a number of processed milks, that is, RO, UF, VE, and a product made from milk with added SMP. The chemical composition of the milk bases is illustrated in Table 2.8 and their conclusions can be summarised as follows:

Table 2.8 Chemical composition (g 100 g⁻¹) of yoghurt milks concentrated/fortified by different methods

Treatment	Total Solids	Fat	Protein	Lactose ^a	Ash
Abrahamsen and Holmen (1980)					
Control	11.84	3.43	3.12	4.45	0.84
VE	14.57	3.49	4.12	6.03	0.93
UF	14.13	3.60	4.97	4.63	0.93
RO	14.54	3.53	4.03	6.07	0.92
SMP	14.32	3.32	4.14	5.93	0.93
Tamime <i>et al.</i> (1984) ^b					
SMP	15.96	1.56	5.55	7.64	1.21
VE	15.11	1.62	5.24	7.11	1.15
UF	11.82	1.55	5.14	4.27	0.84
RO	15.79	1.60	5.51	7.53	1.15
Na-cn	12.87	1.53	5.36	5.13	0.85
Becker and Puhan (1989)					
SMP	13.79	3.50	3.72	5.68	0.89
VE	13.80	3.50	3.71	5.70	0.89
UF	13.71	3.50	4.09	5.13	0.85

^a Figures for lactose (Abrahamsen and Holmen, 1980; Tamime *et al.*, 1984) were calculated by difference. ^b Data is average using three different starter cultures.

VE: vacuum evaporated; UF: ultrafiltration; RO: reverse osmosis; SMP: skimmed milk powder and Na-cn: sodium caseinate.

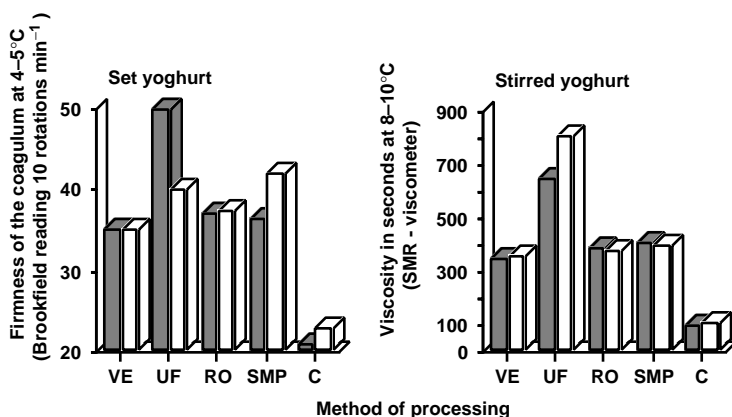


Fig. 2.7 Rheological properties of yoghurts manufactured from milk concentrated/fortified by different methods VE: vacuum evaporation; UF: ultrafiltration; RO: reverse osmosis; SMP: skimmed milk powder addition and C: control (liquid milk)

▨ One day old at 4°C. □ After 14 days storage at 4°C.

Adapted from Abrahamsen and Holmen (1980).

- UF and Na-caseinate yoghurts gave the highest reading for viscosity and firmness of the coagulum (see Fig. 2.7), but the mouthfeel of the latter product was not acceptable (Tamime *et al.*, 1984).
- The favourable instrumental assessment of UF yoghurt was not supported by the organoleptic appraisal and the yoghurt prepared from VE milk proved the most popular (Abrahamsen and Holmen, 1980); however, such effects may not be observed if fruit and sugar are added to the yoghurt as reported by Romero Estevez (1988), Goh *et al.* (1990) and Biliaderis *et al.* (1992).
- The method of fortification can influence dramatically the chemical constituents of the yoghurt mix, but a target figure of about 5g protein 100g⁻¹ is highly recommended.

However, the quality of yoghurt made from milk fortified by the different methods has been reported by many researchers. For example, Na-caseinate, whey proteins and UF milk were used to make yoghurt where the lactic acid content, level of D-lactate, firmness and sensory properties were influenced with the method of fortification used (Renner and Eiselt-Lomb, 1985a-c). In Canada, comparative studies on yoghurt made from milk fortified with different milk proteins (Modler and Kalab, 1983; Modler *et al.*, 1983) suggest the following:

- The gel strength and syneresis were influenced by the method of fortification used and level of casein.
- The casein to non-casein ratio varied between 1.08:1 and 4.56:1.
- The micellar structure of the yoghurt, i.e. fusion of casein micelles, size of micelle chain and flocculated milk proteins, was influenced by the type of milk protein used (e.g. UF milk, caseinate, SMP and WPC using ion exchange, UF or electrodialysis).
- Sensory properties were influenced by method of fortification used.

During the preparation of the milk base, it is probable that a number of different dairy ingredients will be used and it is essential that the levels of SNF and fat are

calculated properly in order to achieve a balanced yoghurt milk. Two approaches can be considered: (i), an approximate formulation can be worked out by the Pearsons Square formula or, (ii), an algebraic method can be used to calculate exactly the quantities of fat and SNF that will be obtained from the various raw materials (Hyde and Rothwell, 1973). The former method of calculation is most satisfactory for small-scale yoghurt producers, but the algebraic method is usually recommended for large-scale manufacture, especially when considering the economics of the operation. Hypothetical examples of the above two methods of calculation are shown in Appendix IX.

2.5 Addition of stabilisers/emulsifiers

2.5.1 General background

Stabilisers and/or emulsifiers are used during the manufacture of some dairy products, but in yoghurt making only stabilisers are added to the milk base. Their application in most countries is governed by legislative regulation. At the international level, the FAO/WHO (1990) have drafted a list of compounds (with permitted concentrations) which can be used in the production of yoghurt and a similar approach has been adopted in the United Kingdom (Statutory Instruments (SI), 1995).

The classification of these food-grade stabilisers/emulsifiers has always proved something of a problem and a number of different schemes have been suggested, such as:

- All compounds to be referred to as polysaccharide materials
- The name to include the botanical origin
- Their general origin, i.e. plant, animal or synthetic
- Chemical grouping.

However, the latter approach has been modified by Glicksman (1969, 1979, 1982, 1983, 1985, 1986) and his proposed classification includes a reference to the processing technique, for example:

- Natural gums (those found in nature)
- Modified natural or semi-synthetic gums (i.e. chemical modifications of natural gums or gum-like materials)
- Synthetic gums (those prepared by chemical synthesis).

Some stabilisers permitted by FAO/WHO (1990) and SI (1995) are illustrated in Table 2.9 and, for convenience, Glicksman's method of classification has been used for the arrangement of the various product groups.

The primary aim of adding stabilisers to the milk base is to enhance and maintain the desirable characteristics in yoghurt, for example, body and texture, viscosity/consistency, appearance and mouthfeel. Thus, the yoghurt coagulum is often subjected to mechanical treatment during manufacture: (a) stirring of the coagulum in the fermentation tank at the end of the incubation period or for in-tank cooling, (b) pumping of the coagulum to a plate/tubular cooler, (c) mixing to incorporate the fruit/flavours into the coagulum, followed by pumping to the filling/package machine, and (d) subsequent postfermentation heat treatment of the coagulum for the manufacture of pasteurised, UHT or long-life yoghurt and as a result the

Table 2.9 Classification and functions of gums which could be used during the manufacture of yoghurt

Natural	Modified	Synthetic ^a
Plant	Cellulose derivatives (1) ^b	Polymers
Exudates	Carboxymethylcellulose	Polyvinyl derivatives
Arabic (1, 3) ^b	Methylcellulose	Polyethylene derivatives
Tragacanth (1) ^b	Hydroxyethylcellulose	
Karaya ^b	Hydroxypropylcellulose	
Extracts	Hydroxypropylmethylcellulose	
Pectins (2, 3) ^b	Microcrystallinecellulose	
Seed flour	Microbial fermentation	
Carob (1) ^b	Dextran	
Guar (1) ^b	Xanthan (1, 3) ^b	
Seaweeds	Miscellaneous derivatives ^b	
Extracts	Low-methoxy pectin	
Agar (2, 3) ^b	Propylene glycole alginate	
Alginates (1, 2, 3) ^b	Pregelatinised starches	
Carrageenan (2, 3) ^b	Modified starches	
Furcelleran (1, 2, 3) ^b	Carboxymethyl starch	
Cereal starches (1, 2, 3)	Hydroxyethyl starch	
Wheat	Hydroxypropyl starch	
Corn		
Animal		
Gelatin ^b		
Casein		
Vegetable		
Soy protein		

^a Limited in their application in yoghurt. ^b Stabilisers permitted by FAO/WHO (1990), and the permitted level (singly or combination with others) is 5 g kg⁻¹, except for pectin, gelatin and/or starch derivatives where it is 10 g kg⁻¹.

Figures in parentheses indicate the function of the hydrocolloid: (1) thickener, (2) gelling agent and (3) stabiliser. The permitted level of these stabilising compounds is specified by the legislative regulations and they are not permitted in natural or unflavoured fermented milks.

Data compiled from Powell (1969), Glicksman (1969, 1979, 1982, 1983, 1985, 1986), Pedersen (1979), FAO/WHO (1990), Baird and Pettit (1991) and Gordon (1992).

yoghurt may become less viscous or, in extreme cases, may show whey separation: The addition of stabilisers can overcome these defects.

Stabilisers are sometimes referred to as hydrocolloids and their mode of action in yoghurt includes two basic functions: first, the binding of water and second, promotion of an increase in viscosity (Boyle, 1972; Thomas, 1982; Morley, 1984; Frost *et al.*, 1984; Phillips *et al.*, 1986, 1992, 1994; Rother, 1994; Doreau, 1994). Thus, the molecules of a stabiliser are capable of forming a network of linkages between the milk constituents and themselves, due to the presence of a negatively charged group, for example, hydrogen or carboxyl radical, or to the presence of a salt possessing the power to sequester calcium ions. These negative groups are concentrated at the interfacial areas and according to Boyle (1972), Dexter (1976), Ingenpass (1980), Baird and Pettit (1991), Kasapis *et al.* (1992), Gordon (1992) and Pedersen (1995), the binding of water into the milk base is achieved by the stabiliser as follows:

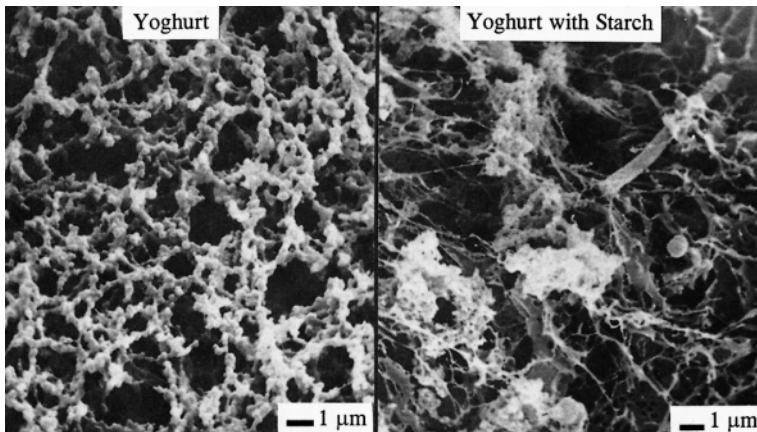


Fig. 2.8 Microstructure of yoghurt with or without added starch as shown by scanning electron microscopy (SEM)

After M. Kalab (personal communication).

- It binds the water as water of hydration;
- It reacts with the milk constituents (mainly the proteins) to increase their level of water hydration;
- It stabilises the protein molecules in the form of a network that retards the free movement of water (see Fig. 2.8 and 2.9).

Therefore, the functions of hydrocolloids in yoghurt are as: (a) gelling or thickening agents, and (b) stabilising agents (Rizzotti *et al.*, 1984; Schaffer, 1989; Thygesen, 1990). Table 2.9 shows the wide range of compounds which can be added to milk for the production of a viscous yoghurt and these stabilisers can be added as single compounds or as a blend. The latter approach is more widely used, since most commercial preparations are a mixture of stabilising compounds (unless it is declared otherwise). The object of blending these compounds together is to achieve a specific function or, in the majority of cases, to overcome one of the limiting properties associated with a specific compound. For example, a single stabilising compound (X) may be suitable for the manufacture of a fruit/flavoured yoghurt, but it may not be suitable on its own for the production of frozen, dried or pasteurised yoghurt. Hence the choice of a particular type of stabiliser is dependent on a multitude of factors, including those in the following sections.

2.5.2 Miscellaneous properties and conditions

2.5.2.1 Functional properties

These include the effect and/or mode of action of the selected stabiliser compound(s), and have to be considered in relation to the type of yoghurt produced. However, in most applications the rule of thumb is trial and error.

2.5.2.2 Optimum concentration

The optimum concentration of stabiliser(s) to be used in yoghurt is sometimes governed by legislation and/or side effects, that is, appearance or undesirable

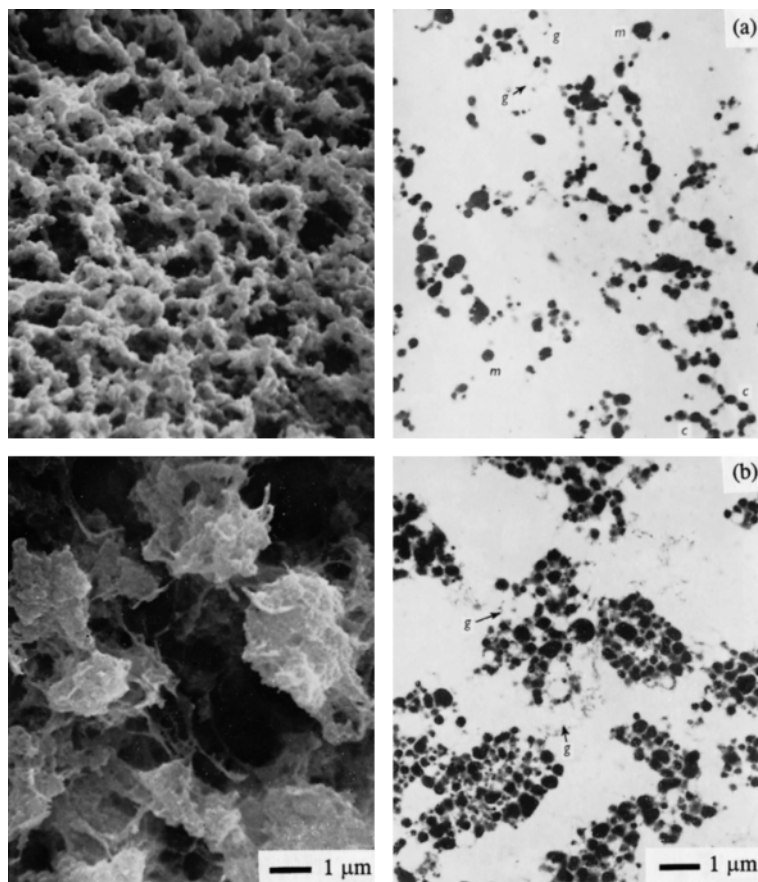


Fig. 2.9 Differences in the microstructure of yoghurt in the presence of different stabilising agents

Micrographs: (left-hand side) scanning electron microscopy (SEM), (right-hand side) transmission electron microscopy (TEM).

(a) Yoghurt supplemented by 0.4% carrageenan and (b) supplemented by 2% pregelatinised waxy maize starch.

The addition of carrageenan resulted in the formation of a fibrillar microstructure which connected large clusters of casein micelles. It can be observed that the fibres had no free terminations, but were thin and long. The presence of starch gave rise to short fibres and sheets and the fibres frequently had free terminations where some of them were connected to small clusters of casein micelles.

TEM showed no differences between the microstructure of yoghurt with starch or carrageenan stabilisers; however, SEM could be used to detect different additives in yoghurt.

Note: (g) fat globules, (f) flat fibres, (h) sheets, (m) casein micelles, magnification 6000 \times .

After Kalab *et al.* (1975). Reproduced by permission of *Journal of Dairy Research*.

mouthfeel, which could be caused by the addition of too large a quantity. Some recommended levels of stabiliser for the manufacture of yoghurt are:

- 0.02–0.7 g 100 g⁻¹ of pectins or some modified starches (Winterton and Meiklejohn, 1978; Zmarlicki *et al.*, 1977; Gudnason *et al.*, 1983; Kratz *et al.*, 1989; Pedersen, 1993; Basak and Ramaswamy, 1994) or 1.2 g 100 g⁻¹ carrageenan and 0.25 g 100 g⁻¹ pectin (Petersen, 1989).

- 0.05–0.6 g 100 g⁻¹ of agar-agar, locust (carob) gum, guar gum, alginate, gelatin, carrageenan or carboxymethyl cellulose (Volker, 1972; Schrieber, 1973; Ledder and Thomasow, 1975; Steinitz, 1975; Hannigan, 1982; van Coillie, 1989; Gonc *et al.*, 1994, Anon., 1995a; Sta, 1996); however, according to Fischer (1996), only high bloom gelatin should be used in yoghurt making due to improved gelatin/casein interactions, its higher melting point and stabilising ability.
- 1–2 g 100 g⁻¹ of some starch preparations (Thomasow and Hoffmann, 1978; Chawal and Balachandran, 1986; Katz, 1991).
- 0.1–0.5 g 100 g⁻¹ guar gum in an acidified milk sample (i.e. 0 to 20 g fat 100 g⁻¹ and 6 to 12 g SNF 100 g⁻¹) did not affect the partition coefficients of acetaldehyde, ethanol or diacetyl (Lo *et al.*, 1996).
- 0.5–2 g 100 g⁻¹ sugarbeet fibre improved the consistency of yoghurt and also gave an acceptable flavour (Saldamli and Babacan, 1997).
- 0.6 g 100 g⁻¹ tapioca-based starch was able to replace 2 g 100 g⁻¹ SNF (i.e. significant cost saving) without affecting the properties of yoghurt; 0.3 gelatin was less effective when compared with starch, especially in yoghurt containing 1.5 g fat 100 g⁻¹ (McGlinchey, 1995, 1997); alternatively, a mixture of cooked wheat grains and sucrose has been used to improve the nutritional and organoleptic properties of yoghurt (Hamzawi and Kamaly, 1992).

Another factor which determines the level of stabiliser added to the yoghurt milk is the percentage of milk solids present. According to Hall (1975), the optimum concentrations (g 100 g⁻¹) for a gelatin/plant gum mixture were 0.5, 0.45, 0.4, 0.3 and 0.25 to yoghurt milks containing 12.5, 14.5, 16.5, 19.0 and 22.0 milk solids, respectively. Other recommended concentrations (g 100 g⁻¹) of stabiliser blends for the manufacture of yoghurt are: (a) 0.35 Gelodan (Mehanna and Mehanna, 1989), (b) 0.3 Na-alginate or gelatin + <1.5 starch (Jogdand *et al.*, 1991a, b), (c) 1 gelatin + 0.2 agar (Ajam *et al.*, 1993), (d) 0.2 Na-alginate + 0.1 β -cyclodextrin (Jiang *et al.*, 1995), (e) 0.06 carboxymethyl guar gum or leucaena gum + carrageenan at a ratio of 9:1 (El-Etriby *et al.*, 1994; Abd El-Salam *et al.*, 1996), (f) polymer solution of locust bean gum and carrageenan (Arnaud *et al.*, 1989), and (g) 2.0 Gelodan YF 358 (fibre + milk proteins) and 1.5 Gelodan YF 326 (fibre + gelatin) or 1.5 Gelodan YF 314 (fibre + milk proteins + low methoxyl pectin) and 1.5 Gelodan 361 (fibre + gelatin + low methoxyl pectin) (Carnell, 1989). However, studies evaluating different stabilisers during the manufacture of yoghurt have also been reported by Jamrichova (1985, 1990), Shukla *et al.* (1988), Shukla and Jain (1991) and Jawalekar *et al.* (1993).

2.5.2.3 Toxic or inhibitory effects

Some stabilisers, for example carrageenans, tragacanth and locust (carob) gum, are still awaiting toxicological clearance for use in foodstuffs, but in general, stabilising compounds do not inhibit the yoghurt organisms at the rates normally employed.

2.5.2.4 Legal aspects

These may differ with the country concerned and not all stabilising compounds are permitted for the production of yoghurt; hence, statutory regulations should be checked on a regular basis.

2.5.2.5 Solubility and dissolution

The solubility and dissolution of some starch preparations and Na-carrageenan are at an optimum at low temperature and hence they can be added to cold milk during the preparation of the milk base. The majority of the stabilising compounds are, however, only soluble at higher temperatures, for example, 50–85°C (with the exception of agar-agar at 90–95°C), so that in practice these stabilisers are added to warm milk before pasteurisation, or alternatively to hot milk after the heat treatment. In some instances, complete dissolution of a particular stabiliser blend, for example, one which contains a starch preparation, may necessitate a holding time at high temperature in order for the mixture to become active as a stabiliser.

In view of the different properties of these compounds, it is difficult to recommend one method for incorporation into the milk base, but the following points may help to overcome any problems:

- Follow the instructions provided by the manufacturer or in the absence of any information: (a) mix the stabiliser with the milk powder and add to the water or milk with high speed stirrer at the temperature recommended for the milk powder, or (b) mix the stabiliser with the sugar and add to the milk base under high speed agitation at the temperature recommended for the sugar.
- Hydrate the stabiliser (e.g. gelatin powder) in water or milk and then add to the basic mix with high speed stirring.

2.5.2.6 Casein

The effect on the casein of some hydrocolloids (Na-carboxymethyl cellulose, guar gum and locust bean gum) at levels as low as 0.05 g 100 g⁻¹ in sweet milk can destabilise the casein micelle (Powell, 1969) and, although the destabilised casein micelles will eventually coagulate, the matrix has a rather limited ability to retain water and syneresis becomes evident. Furthermore, such destabilised casein can give rise to a coarse coagulum with an open texture. The problem can be minimised, however, by blending the above compounds with carrageenan or alginates (see also Dexter, 1976).

2.5.2.7 Processing conditions

The processing conditions for various yoghurt-based products have been developed (see Chapter 5) and the success of these is dependent on the addition of stabilisers. For example:

- Pasteurised, UHT or long-life yoghurt – it is recommended that a gelling agent is added consisting of a blend of locust gum and agar-agar and/or xanthan (Anon., 1980a); the presence of starch derivatives (diamylopectin glycerol ether or diamylopectin phosphate) can improve the appearance of heat-treated yoghurt (Vanderpoorten and Martens, 1976).
- Frozen yoghurt – an unspecified mixture of stabilisers/emulsifiers is recommended by Gautneb *et al.* (1979), but the addition of modified starch proved unsatisfactory (Winterton and Meiklejohn, 1978).
- Stirred yoghurt – a blend of (g 100 g⁻¹) 1 Na-proteinate (possibly Na-caseinate), 0.1 Frimulsion J5, 0.1 Genu gum CH 200, 0.3 Genu carrageenan with maltodextrin or 0.16 Frimulsion JQ improved the viscosity of the product (Luczynska *et al.*, 1978).

- Drinking yoghurt – an agar-agar based stabiliser is added at a rate of $0.25\text{ g }100\text{ g}^{-1}$ and this helps to maintain the suspension of fruit in the product (Morley, 1978).
- Freeze-dried dahi – the quality of the product was improved by the addition of corn starch and lecithin or glycerol monostearate to the fermented milk prior to drying (Baisya and Bose, 1975).

Since casein precipitation may occur in sweet milk or during the development of acid, some of the stabilisers may be added to the yoghurt after the formation of the coagulum. In this case it is recommended that the stabilising compound (e.g. liquefied agar-agar and/or preswollen gelatin) is mixed with the sugar and then incorporated into the coagulum. Refer to Chapter 5 for further details and up-to-date information regarding the use of stabilisers in yoghurt-related products.

2.5.2.8 *Solidification characteristics*

The majority of stabilisers used in the production of yoghurt will exhibit solidification characteristics at ordinary refrigeration temperature, with the exception of gelatin and agar-agar which solidify at 25°C and $42\text{--}45^{\circ}\text{C}$, respectively. These latter stabilising compounds can, therefore, cause problems during the cooling stage, i.e. difficulty in pumping and/or packaging and, in addition, the use of gelatin may give the coagulum a rough texture. This latter fault can be reduced or eliminated by passing the coagulum through a fine mesh screen or sieve.

2.5.2.9 *Hygienic standards*

It is recommended that suitable hygienic standards be applied to the stabilisers. However, the temperature used during the processing of the yoghurt milk (85°C for 30 min or $90\text{--}95^{\circ}\text{C}$ for 5–10 min) is high enough to destroy the majority of micro-organisms which could be present in the stabiliser. Stabilisers added to the coagulum after the incubation period must be of excellent microbiological quality, otherwise the shelf life of the product could be reduced.

2.6 Addition of sweetening agents

2.6.1 General introduction

Sweetening compounds are normally added during the manufacture of fruit/flavoured yoghurt and, in some instances, for the production of “sweet” natural yoghurt; the latter product is of limited demand.

The main object of the adding sweetening agents to yoghurt is to tone down the acidity of the product and the level of incorporation is dependent on:

- type of sweetening compound used,
- consumer preference,
- type of fruit used,
- possible inhibitory effects on the yoghurt starter organisms,
- legal aspects, and/or
- economic considerations.

On average, fruit/flavoured yoghurts may contain as high as $20\text{ g }100\text{ g}^{-1}$ carbohydrates and these are derived from: (a) residual milk sugars (lactose, galactose and

glucose) – the level varies in relation to the level of solids in the milk base and the method of fortification, (b) natural sugars present in the fruit (sucrose, fructose, glucose and maltose) and (c) sugars added by the yoghurt manufacturer and/or the fruit processor.

Fruit may contain different levels and types of natural carbohydrate and the total content ranges from as low at 1.6 g 100 g⁻¹ in lemon to as high as 65 g 100 g⁻¹ in raisins (Shallenberger and Birch, 1975; Holland *et al.*, 1991). The fruits, which are in regular demand, have the following natural carbohydrate content (g 100 g⁻¹):

Apricot	7.5
Black cherry	12.0
Blackcurrant	6.6
Mandarin	14.2
Peaches	9.0
Pineapple	11.6
Raspberry	5.6
Strawberry	6.2

The main carbohydrates present in fruits are glucose, fructose, sucrose and maltose, and hence the perceived sweetness of each type of fruit is dependent on the level and type of carbohydrate present. The comparative sweetness of various carbohydrates, including milk sugars and synthetic sweeteners, is illustrated in Table 2.10; sucrose is given a nominal rating of one.

The fruit preparations which are utilised by the yoghurt industry may be divided into two main categories, fruit preserves which do not contain any added sweeten-

Table 2.10 List of various sweetening compounds

Sweetening compound	Relative sweetness: sucrose = 1
Lactose	0.4
Dulcitol	0.4
Maltose	0.4
Sorbitol	0.5
Mannose	0.6
Galactose	0.6
Glucose	0.7
Xylose	0.7
Mannitol	0.7
Glycine	0.7
Invert sugar	0.7–0.9
Glycerol	0.8
Sucrose	1.0
Fructose	1.1–1.5
Cyclamate	30–80
Acesulfame K	150–200
Aspartame	200
Saccharin	240–350
Neohesperidin DC	1500–2000
Thaumatococin	3000

After: Beck (1974), Crosby and Wingard (1979), Shallenberger and Birch (1975), Meade and Chen (1977), von Lipinski and Klein (1989), Canales (1991), von Lipinski (1990) and Stasell (1993).

ing agent and fruits with added sweeteners. The latter type is more popular and the level of added sweeteners in processed fruits for yoghurt manufacture ranges from 25 to 65 g 100 g⁻¹, with the most popular level being 30–35 g 100 g⁻¹ (J.G. Spinks, personal communication).

It is now almost universal practice to add preserves and similar materials to the finished yoghurt, since the presence of carbohydrates in the milk base can inhibit the growth of the yoghurt organisms. Thus, Tramer (1973) reported a reduction in the rate of acid development by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in concentrated milk (16.5 g TS g 100 g⁻¹) as the sugar level was increased from 6 to 12 g 100 g⁻¹, and a microscopic examination of these different types of yoghurt showed that first, *S. thermophilus* was more tolerant of high sugar concentrations than *L. delbrueckii* subsp. *bulgaricus* (a view which was confirmed by Steinsholt and Abrahamsen, 1978; Marshall and Mabbitt, 1980) and second, that morphological changes occurred, that is, the cells were distorted, elongated and “unhealthy looking”. Kim *et al.* (1995) reported that the use of >9 g 100 g⁻¹ sugar in the milk base reduced the rate of acid development and decreased the viscosity of the yoghurt (see also Coghill, 1983; Grandi and Lopes-Andrade, 1989; Latrille *et al.*, 1992; Cislighi *et al.*, 1995). However, in Finland, strawberry yoghurt containing 3.5 g fat 100 g⁻¹ and sucrose 10 g 100 g⁻¹ was highly rated by the male panellists, but not the female, and such observation may be used to segment consumers in order to predict product success (Tuorila *et al.*, 1993). It was evident, however, that the sugar tolerance of the starter cultures was strain dependent and it was recommended that the strains of starter culture to be employed in presweetened milks should be carefully screened.

Commercially available starter cultures are tolerant of sugar levels up to 12 g 100 g⁻¹ in the milk base, but recently one such culture showed a slight delay in the fermentation period (i.e. about 30 min) when grown in milk containing 9 g sugar 100 g⁻¹ (Tamime, unpublished data). However, in a recent study in Korea (Song *et al.*, 1996), the growth of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was inhibited by the following concentrations (g 100 g⁻¹) of sweeteners: sucrose ≥4, fructose ≥2.7, aspartame ≥0.02, fructo-oligosaccharide ≥7.3 and isomalto-oligosaccharide ≥7.7.

The inhibition of yoghurt starter cultures in milk (14–16 g TS 100 g⁻¹) plus added sugar (10–12 g 100 g⁻¹) is due mainly to the adverse osmotic effect of the solutes in the milk, but low water activity (Shallenberger and Birch, 1975; Labuza, 1980) may also be involved. The water activity (A_w) of a food is described as:

$$A_w = \frac{P_f}{P_o} = \frac{\text{ERH}}{100}$$

where A_w = water activity, P_f = vapour pressure of water in food, P_o = vapour pressure of pure water at the same temperature and ERH = equilibrium relative humidity.

This latter concept is important from a quality control point of view, since both microbial growth and enzyme activity in foods are related to the A_w (Acker, 1969), and hence it is possible to suggest that both osmotic pressure and A_w may be associated with the inhibitory effect on yoghurt starter organisms. However, starter cultures propagated in milks with high total solids, for example, 30 g TS 100 g⁻¹, can also show reduced activity (Zmarlicki *et al.*, 1974), a condition which could be entirely related to the A_w of the growth medium. This observation was also reported by

Tramer (1973), who observed the inhibition of yoghurt starter cultures propagated in milk (21 g TS 100 g⁻¹) plus 3 g 100 g⁻¹ added sugar; the inhibitory effect was attributed to A_w , since it was considered unlikely that 3 g sugar 100 g⁻¹ in solution could create enough osmotic pressure to retard the growth of the organisms.

In view of the above data, the normal methods used for the addition of sweetening agents are as follows: (a) the yoghurt manufacturer adds up to 5 g 100 g⁻¹ sweetener (sugar) to the milk base and (b) the sweetness desired in the final product is attained by the addition of a sweetened fruit preparation.

It is worthwhile pointing out at this stage that the sugar content of frozen yoghurt is much higher than in ordinary fruit/flavoured yoghurt. It is recommended that the quantity of sugar (sucrose) added to the milk base should not exceed 10 g 100 g⁻¹, with the balance being added to the cold yoghurt prior to freezing. Different types of carbohydrate may be used during the manufacture of sweetened fruit/flavoured yoghurt and some examples of these are given in the following section.

2.6.2 Types of carbohydrate sweetener

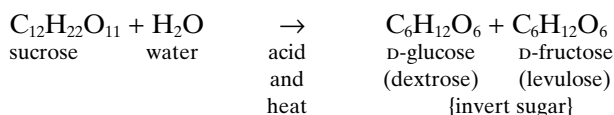
2.6.2.1 Sucrose (saccharose)

Sucrose is abundant in the plant kingdom and it is normally referred to as sugar. Sucrose has the empirical formula $C_{12}H_{22}O_{11}$ and the refined carbohydrate is obtained commercially from sugar cane or sugar beet. It is widely used in the food industry as a sweetening agent and can be obtained in a granulated or syrup form. The former type requires strong agitation/stirring for complete dissolution when added to liquid milk and, in practice, it is added with the rest of the dry ingredients at around 40°C. The syrup type, which contains 65–67 g 100 g⁻¹ sugar (saturated at 20°C), is easily mixed with the aqueous phase of the milk base but, since it contains 33–35 g 100 g⁻¹ moisture, it dilutes the level of solids in the yoghurt milk, and this added water must be allowed for when calculating a balanced mix.

The addition of sugar before the heat treatment of the milk is highly desirable, since it ensures the destruction of any vegetative contaminants, for example, osmophilic yeasts and moulds. However, if the sugar has to be added after the formation of the coagulum, steps must be taken to avoid uneven distribution of the sugar and excessive reduction in the consistency of the product.

2.6.2.2 Invert sugar

This type of carbohydrate results from the “inversion” of a sugar with dextrorotatory optical activity to one that is laevorotatory or vice versa. The different types of invert sugar depend on the raw material. For example, invert sucrose syrup is formed when sucrose undergoes acid hydrolysis in the presence of heat and the degree of inversion can range from 10 to 90%.



One advantage of this conversion is that a product (50% inversion) contains 23 g 100 g⁻¹ moisture (Junk and Pancoast, 1973) and yet can be handled at this high sugar concentration without crystallisation. However, invert corn syrup is formed by the

hydrolysis of corn starch with the production of D-glucose (dextrose) and the degree of conversion is measured in terms of dextrose equivalent (DE), that is, types I (20–37 DE), II (38–57 DE), III (58–72 DE) and IV (>73 DE) (Junk and Pancoast, 1973). The process of hydrolysis is normally achieved by one of these methods:

- total acid hydrolysis
- acid liquefaction/enzyme saccharification
- total enzyme hydrolysis

In recent years starch syrups have also been processed into other types of sugars, for example, syrups high in maltose or fructose. The latter syrup has many potential applications in the food industry and according to Martin (1979), high fructose (corn) syrups are commercially produced in the United States containing 42 g, 55 g or 95 g fructose 100 g⁻¹; the corresponding sucrose equivalents, in terms of sweetness (sucrose = 1), are 1, 1.1–1.2 and 1.5, respectively (see also Dordovic *et al.*, 1981).

2.6.2.3 Fructose (*laevulose*)

Fructose or fruit sugar has the same empirical formula as glucose, C₆H₁₂O₆, and as can be seen from Table 2.10 is sweeter than both sucrose and glucose. Commercially, fructose is derived mainly from the conversion of starch, but recently, grape must containing fructose has been used at a rate of 20 g 100 g⁻¹ to sweeten yoghurt (Calvo *et al.*, 1995).

2.6.2.4 Glucose (*dextrose*)

Glucose has the same empirical formula as fructose, C₆H₁₂O₆, and is commercially produced from the hydrolysis of corn starch.

2.6.2.5 Glucose/galactose syrup

This is produced from whey, a by-product of the cheese and casein industries, and of the permeate of UF concentrated milk. The amount of lactose in whey is usually in the region of 5 g 100 g⁻¹ but, as illustrated in Table 2.10, the relative sweetness of lactose is only 0.4 compared with sucrose; hence the lactose has to be converted to its monomer constituents – glucose and galactose – before it can impart any real sweetness (see Table 2.10). The process of hydrolysis of lactose can be achieved using either acid or enzymes. The review by Sienkiewicz and Riedel (1990) provides details of these processes, the chemical composition of the different syrups and characterisation of the enzymes used, including commercial preparations and their countries of origin.

2.6.2.6 Miscellaneous sweeteners

Sorbitol is an alcohol, produced commercially from glucose by a hydrogenation process, that is, the aldehyde group (CHO) in the glucose molecule is converted to an alcohol group (CH₂OH). Although sorbitol has only half the sweetness of sucrose (see Table 2.10), it has a possible application in fruit/flavoured yoghurts for patients suffering from diabetes. Thus, the rate of absorption of sorbitol in the gut is slower than that of glucose and hence has little effect on the level of sugar in the blood. No recommended daily intake is given, since large intakes cause diarrhoea (Davidson *et al.*, 1979).

Saccharin and cyclamate are artificial sweeteners and their sweetness compared with sucrose is 240–350 and 30–80, respectively (Table 2.10). However, due to

Table 2.11 Reported characteristics of yoghurt made with artificial sweeteners

Sweetener (g 100 g ⁻¹)	Comment	Reference
Xylitol 8, fructose 7, cyclamate 0.07 and xylitol 4 + saccharin 0.007	Xylitol retarded the starter culture growth and was only suitable when used with sucrose; the rates used were satisfactory alternatives to 8 g sucrose 100 g ⁻¹ .	Hyvonen and Slotte (1983)
Thaumatococin 0.0002–0.0003 or up to 0.1	This is a protein sweetener derived from the fruit <i>Thaumatococcus daniellii</i> and has been used in Japan.	Ohashi and Ochi (1983), Yasuda <i>et al.</i> (1985)
Aspartame 0.1–0.75	Sweetener was mixed with a stabilising solution (g 100 g ⁻¹) consisting of: 3–8 low methoxyl pectin, 2–7 high methoxyl pectin and 0.2–2.5 Na-hexametaphosphate; the mixture was pasteurised and added to yoghurt at a ratio of 1:3–7.5 (v/v).	Malone and Miles (1984)
Aspartame 0.14	The added rate was equivalent to 2 g sucrose 100 g ⁻¹ , but 64% of panellists preferred sucrose because of the lingering aftertaste and slow development of sweetness when aspartame was used.	Greig <i>et al.</i> (1985)
High fructose corn syrup 4	Swiss-type yoghurt sweetened with fructose syrup (<i>c.</i> 90 g 100 g ⁻¹) was highly rated ($P \leq 0.001$) especially in the strawberry product; acetaldehyde and diacetyl contents were not influenced by the sweetener, but acetone content was highest in the experimental yoghurt.	Wilson-Walker (1982), McGregor and White (1986, 1987)
Aspartame 0.02 or sorbitol 7.4 plus polydextrose	Nine different sweeteners were evaluated, but sorbitol and aspartame were highly favoured; slight decrease in flavour was detected after 42 day storage period, but not after 28 days.	Keating and White (1990), White (1991)
NutraSweet®	Typical recommended quantities used were 400–500 mg l ⁻¹ for fruit yoghurt or 700 mg l ⁻¹ for mocha yoghurt; in France and India, low fat yoghurt sweetened with aspartame had high scores for overall preference.	Wiese (1988), Dupont (1989), Kumar and Atmaram (1991)

Sweetener (g 100 g ⁻¹)	Comment	Reference
Actilight®	This sweetener consisted of fructo-oligosaccharide 1-kestose, nystose and fructosyl nystose; this product stimulated the growth of lactobacilli and bifidobacteria.	Thiriet (1989)
Aspartame	Stability of this sweetener in fruit preparation was 1½, 4–6 or >6 months at 32.2°C, 21.1°C and 4.4°C, respectively; the stability in sundae-style yoghurt (i.e. with fruit in the bottom) was good.	Fellows <i>et al.</i> (1991a, b), Saldamli <i>et al.</i> (1991)
Different sweeteners	Yoghurt made with acesulfame remained stable during the storage period whilst the product sweetened with aspartame degraded slightly during the fermentation period, but was stable during storage.	Lotz <i>et al.</i> (1992)
Aspartame and acesulfame-K	Sensory tests on yoghurt revealed synergistic effects between these sweeteners, but no effect on the textural properties; recommended level of each type of sweetener in strawberry yoghurt was 0.016 g 100 g ⁻¹ .	Lotz <i>et al.</i> (1993), Anon. (1996)
Natren 0.3–0.4, aspartame 0.04 or lactose hydrolysed milk	Yoghurt made with natren and raspberry syrup was highly rated by the taste panellists.	Tosovic <i>et al.</i> (1994)
Different mixtures	Mixture of sweeteners (e.g. fructose + aspartame or fructose + aspartame + acesulfame) gave the highest sweetness intensity in yoghurt and the cost was lowest.	Rollet (1995)
Neohesperidine	This sweetener alone or in combination with acesulfame was stable in yoghurt after storage for 6 weeks at 3°C.	Montijano <i>et al.</i> (1995), Montijano (1996), Montijano and Borrego (1996)

possible toxic effects, cyclamate has been banned in many countries as an additive, and although saccharin is still permitted, its use is closely observed by food and drug administrators worldwide. The use of these sweeteners in the food industry is, therefore, restricted. In the present context, it should be noted that some information is available regarding the effect of the above sweetening agents on the activity of yoghurt starter cultures. Thus, Gautneb *et al.* (1979) reported an inhibition of acid production by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* when the yoghurt milk was fortified with a sweetening agent composed of 99.9 g 100 g⁻¹ sorbitol and 0.1 g 100 g⁻¹ saccharin. As a safeguard these types of sweetener should be added after the fermentation of the milk (see also Hyvonen and Slotte, 1983). Table 2.11 illustrates the latest reported information regarding the effect of synthetic sweeteners on the quality of yoghurt, growth characteristics of the starter cultures and stability of the sweetener(s) during the storage period. However, for further technical information on artificial sweeteners (see Hough *et al.*, 1979; Grenby *et al.*, 1983; Grenby, 1987) and relevant data on yoghurt, the reader is referred to reviews by Hugill (1980), Harrison and Bernhard (1984), Homler (1984), van Tornout *et al.* (1985), Billaux (1989), Sasso (1989), Akahoshi *et al.* (1990), Pedersen (1991), Sardesai and Waldshan (1991), Farooq and Haque (1992) and Borrego and Montijano (1997).

Any of these different types of sweetening agents could be employed for the manufacture of fruit/flavoured yoghurts and the choice of any one particular sugar is determined by one or more of the following factors:

- Availability and cost of the sweetening compound: for these reasons it is probable that sucrose is the most widely used.
- Legal aspects: whether a certain sugar is permitted as a food additive, although since most sweetening agents are derived from natural products, with the exception of the artificial sweeteners, prohibition is unlikely.
- Storage facilities: granulated products are stored in multilayer bags or large silos and humidity control in the storage area is essential to prevent “caking”; details of bulk storage requirements are discussed by Junk and Pancoast (1973), Meade and Chen (1977), Kaplinsky (1989) and Chen and Chou (1993); syrups are mainly stored in large metal containers or silos.
- Nutritional aspects: fructose is a very sweet sugar and a sucrose/fructose syrup mixture used at a low level can provide both sweetness and a reduced calorie intake; in addition, fructose, like sorbitol, is absorbed only slowly into the bloodstream and its use in “diabetic” yoghurt production is a clear possibility.

2.7 Addition of miscellaneous compounds

During the preparation of the milk base, some yoghurt manufacturers add compounds to the milk in order to achieve specific objectives. Some examples of such additives are as follows.

2.7.1 Penicillinase

Intramammary injection of antibiotics is widely used for the treatment of mastitis in the dairy cow and residues of these compounds in milk can inhibit the growth of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (see Chapter 6). Relevant data on the structure, mode of action and other related biochemical activity(s) of anti-

microbial drugs can be found in Pratt and Fekety (1986) and Williams *et al.* (1996). Although statutory regulations have been introduced in different countries to limit the level of these inhibitory compounds in milk, even the permitted values can reduce the activity of the yoghurt starter culture. As a result, methods have been sought to inactivate the different antibiotics and notable success has been achieved in the case on penicillin. The inactivation of penicillin is carried out enzymatically using penicillinase (β -lactamase, EC 3.5.2.6), which is contained in the filtrate from different cultures of *Bacillus* species. One such preparation is commercially available under the name Bacto-Penase. β -Lactamase is specific in hydrolysing cyclic amides, that is, β -lactam in penicillin, thus producing an antibiotically ineffective compound; the structure of penicillin and the neutralising action of β -lactamase are illustrated in Fig. 2.10.

The activity of penicillinase preparations can be assayed by chemical or microbiological methods. Results from the former technique are expressed in Levy units (LU) or Kersey kinetic units (KKU), while the microbiological method measures the “units” of penicillin being inactivated. For example, 1 ml of Bacto-Penase has a potency of 2000 LU, 200 000 KKU, or can inactivate 1 000 000 units of penicillin G; the Bacto-Penase concentrate is ten times more active than the standard penicillinase preparation.

In commercial practice, penicillinase is added to the milk with the rest of the dry ingredients and it is recommended that it should be added at ambient temperature; high temperatures, for example, those employed in the heat treatment of yoghurt milk, can inactivate it. However, it is important to note that penicillinase is only effective against penicillin and that it should only be added to milk known to be contaminated with penicillin, a situation which is difficult to determine. Thus, routine

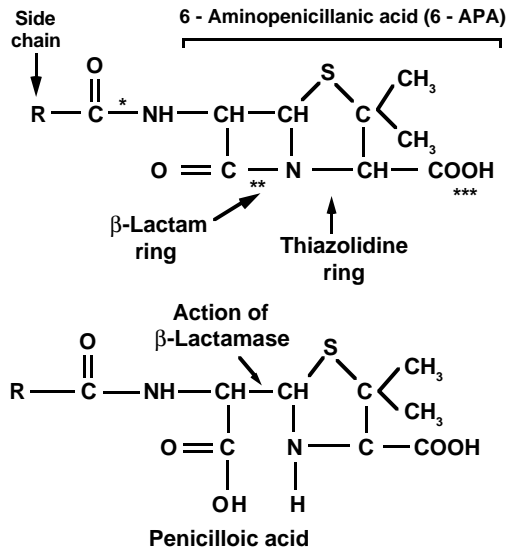


Fig. 2.10 Basic structure of penicillin and the mode of action of β -lactamase

*, Site of action of amidase; **, site of action of β -lactamase; ***, site of salt formation.

The β -lactam ring of the 6-APA is split by the action of β -lactamase to produce a bacteriologically inert penicilloic acid; however, the specific action is reduced or increased by the nature of the side chain.

addition to the yoghurt milk may prove uneconomical in the long run, especially as 60% of the antibiotics used in the United Kingdom for mastitis therapy are not penicillin(s).

Another approach to inactivation of the penicillin content in milk was investigated in the U.K. and Poland, where the yoghurt milk was treated with selected strains of *Micrococcus* spp. (Reiter *et al.*, 1961; Czarnocka-Roczniakowa and Maciejka, 1985). In another study, *Micrococcus* spp. and lactic acid bacteria were inoculated simultaneously, and the latter micro-organisms were able to grow in the presence of low concentrations of penicillin about 0.3 IU ml^{-1} (Maciejka and Czarnocka-Roczniakowa, 1985, 1989).

2.7.2 Preservatives

Different types of preservative are used in the food industry, including the processing of fruits, where they are effective growth inhibitors against yeast and moulds (Restaino *et al.*, 1982; Eklund, 1983; Andres, 1985). The addition of such fruits to yoghurt results in the carry-over of some of these compounds, and hence, in the United Kingdom, for example, the SI (1995) provides general information regarding preservatives which are permitted in fruit yoghurt, but not in natural yoghurt. A similar approach has also been adopted by FAO/WHO (1990) and the permitted preservatives in yoghurt, which come exclusively from the fruit preparations, are sorbic acid (including its Na-, K- and Ca-salts), sulphur dioxide and benzoic acid. The maximum permitted level in the final product is 50 mg kg^{-1} (singly or in combination) (FAO/WHO, 1990).

In view of the fact that preservatives are allowed in fruit yoghurt, some manufacturers are inclined to fortify the milk base with one of the preservatives (e.g. sulphur dioxide, sorbic acid, benzoic acid, benzoates and/or ethyl, methyl or propyl *p*-hydroxybenzoate) in the hope of prolonging the keeping quality of the product. This approach is not, however, one to recommend, partly because the end products may not comply with the statutory regulations of an intended market and partly because the presence of such compounds in the milk may affect the growth of the starter culture. One preservative which might be an exception to this rule and which is widely used in the dairy industry (cheese and cheese products) is sorbic acid.

This compound is commercially available as a powder in the acid form ($\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{COOH}$) or as the potassium or sodium salt ($\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{COOK}$ or Na), that is, potassium or sodium sorbate. These salts are used more commonly than the acid and their antimycotic activity is released at low pH, < 6.5 , where the salt is ionised to produce the free acid (Anon., 1974, 1981b). It should also be noted that K- or Na-sorbates yield only 75% of the inhibitory strength shown by sorbic acid.

For example:

$$0.13 \text{ g } 100 \text{ g}^{-1} \text{ K or Na-sorbate} \cong 0.1 \text{ g } 100 \text{ g}^{-1} \text{ sorbic acid} \cong 1000 \mu\text{g g}^{-1}.$$

Sorbic acid is a mycostatic agent in that it does not reduce the actual number of yeasts and moulds in the product, but merely inhibits their activity, perhaps by interfering with their dehydrogenase systems. The effect of potassium sorbate on the activity of yoghurt starter cultures has been studied by Hamdan *et al.* (1971) and they reported a reduction in growth, acid development and acetaldehyde production. The dose rate of potassium sorbate was 0.05 and $0.1 \text{ g } 100 \text{ g}^{-1}$, which would be

Table 2.12 Effect of potassium sorbate ($C_3H_7O_2K$) on pH values developed by three commercial yoghurt starter cultures (3% inoculation rate) incubated at 45°C

Time of incubation (h)	Starter culture R ₁			Starter culture 403			Starter culture 405		
	A	B	C	A	B	C	A	B	C
	pH								
2	4.75	5.00	5.10	4.75	4.90	5.15	4.85	5.05	5.25
3	4.35	4.60	4.70	4.40	4.50	4.60	4.45	4.65	4.75
4	4.10	4.40	4.50	4.20	4.30	4.40	4.20	4.40	4.50

A, Control, no $C_6H_7O_2K$ added. B, Milk contains $0.05\text{ g }100\text{ g}^{-1}$ $C_6H_7O_2K$. C, Milk contains $0.1\text{ g }100\text{ g}^{-1}$ $C_6H_7O_2K$.

Adapted from Hamdan *et al.* (1971).

equivalent to 375 and $750\mu\text{g g}^{-1}$ of free sorbic acid, respectively. The rate of acid production by three different commercial starter cultures is illustrated in Table 2.12 and it can be observed that, at the lower concentration, the inhibition delayed the processing time by 1 hour.

Oebentraut *et al.* (1982, 1984) reported that 72 and 92 samples of set- and stirred-type yoghurts, respectively, in Austria contained benzoic acid at $14\text{--}16\mu\text{g g}^{-1}$ and $10\text{--}19\mu\text{g g}^{-1}$, respectively. While in Japan two samples of dried yoghurt had benzoic acid contents between 190 and $282\mu\text{g g}^{-1}$ (i.e. equivalent to about 26 and $39\mu\text{g g}^{-1}$ of benzoate in fresh yoghurt), and only one sample contained $233\mu\text{g g}^{-1}$ of sorbic acid (i.e. about $32\mu\text{g g}^{-1}$ in fresh yoghurt) (Arimoto *et al.*, 1987; see also Serrano *et al.*, 1991).

In Turkey, ayran (i.e. a cultured milk beverage) containing $0.06\text{ g }100\text{ g}^{-1}$ of sorbic acid had an extended shelf life of up to 70 days under refrigerated storage (Oysun, 1988). However, although the use of Na-benzoate and K-sorbate at different rates extends the keeping quality of yoghurt and has a minimal effect on the sensory character, in some instances reduced starter culture counts at the end of the storage period have been noted (Sanyal *et al.*, 1990; Rajmohan and Prasad, 1994; Souad *et al.*, 1994). Reviews by Sieber *et al.* (1995) and Horak *et al.* (1997) highlighted the state-of-the-art view of benzoic acid as a naturally occurring preservative in cultured dairy products and cheese and illustrate the possible metabolic pathways for its formation.

An alternative approach to extend the keeping quality of yoghurt is the addition of Nisin which is a natural bacteriocin produced by certain species of *Lactococcus lactis* subsp. *lactis*. The sensitivity of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* has been studied by many researchers. It is safe to conclude that maximum inhibition of the yoghurt organisms occurs in milk containing $100\text{--}200\text{ RU ml}^{-1}$ of Nisin (Naguib *et al.*, 1985a; Lee and Kim, 1985a, b; Gupta and Prasad, 1988, 1989a–c; Kebary and Kamaly, 1991). However, Bossi *et al.* (1989) reported that, while *S. thermophilus* was insensitive to Nisin (i.e. up to 5 RU ml^{-1}), the same concentration inhibited *L. delbrueckii* subsp. *bulgaricus*, a result that suggests strain sensitivity among these strains of lactobacilli. Nisin-producing organisms (i.e. 1000 IU ml^{-1}) were used to produce dahi in India, but the bacteriocin did not inhibit the proliferation of yeasts and moulds during the storage period (Rajmohan and Prasad, 1995a, b).

One preservative for yoghurt, which is likely to become important in the next few years, is natamycin or primaricin as it is often called. It was originally derived from *Streptomyces natalensis* and it is a polyene antibiotic that offers, in the present context, the following attractions:

- It is biocidal against yeasts and moulds, unlike sorbic acid which merely inhibits growth.
- It has no effect on the bacteria of the starter culture.
- It is sufficiently thermostable to withstand addition to the yoghurt milk prior to heating at 95°C for 7–10 min.
- It is sufficiently acid stable to withstand pH 4.0 for two/three weeks.

Delvocid (Gist-Brocades, Delft, Holland) is the commercial form of the fungicide, and each gram contains 500 mg of natamycin. Dosages of 10–20 mg kg⁻¹ of natamycin have been shown to be effective in preventing microbial spoilage of yoghurt for up to 4 weeks and no interference in the fermentation process or the survival of the lactic acid bacteria was observed (Robinson, unpublished data).

As with sorbic acid, the optimum activity of natamycin lies in the acid range, but whereas the activity of potassium sorbate outside this range is limited, that of natamycin is less pH dependent. The activity of natamycin is based on its reaction with ergosterol, a compound in the cell wall membranes of yeasts and moulds. Due to this reaction, the cell membrane disrupts, leading to leakage of intracellular liquids and salts and eventually to death of the cell. As bacteria do not have these sterol-like compounds in their cell wall membranes, natamycin does not exert any antibacterial activity.

At present, natamycin can be used legally to prevent the growth of moulds on the rind of cheese, but it has not received regulatory approval for use in yoghurt. However, this situation may well change, since Brik (1981) states that natamycin is not absorbed from the human intestinal tract even if consumed at a rate of 500 mg day⁻¹ and even an intake of 1000 mg day⁻¹ only produced symptoms of nausea and diarrhoea. The LD₅₀ for the oral administration of natamycin to rats is 1500 mg kg⁻¹ body weight. Obviously it is important that the recommended daily intake (RDI) is only 0.3 mg kg⁻¹ body weight (FAO/WHO, 1990), but it is not clear why the figure is so conservative. Nevertheless, it should be noted that this figure only implies that a man of eleven stone should not consume more than 20 mg of natamycin day⁻¹ and, with a proposed inclusion rate of 15 mg kg⁻¹ of yoghurt, few consumers are likely to exceed the recommended RDI.

Yet other preservatives used in yoghurt making include (a) K-nitrite which inhibits the growth of lactococci and streptococci (Naguib *et al.*, 1985b), (b) nitrite or nitrates reduce acid development and viscosity of yoghurt (Baranova *et al.*, 1996, 1997; see also Steinka and Przybylowski, 1997), (c) lysozyme inhibits the growth of lactobacilli but not *S. thermophilus* (Kontova and Prekoppova, 1990) and (d) Microgard™ can inhibit the growth of yeasts and moulds in yoghurt at concentrations ranging between 0.5 and 10 g 100 g⁻¹ (Weber and Broich, 1986; Salih and Sandine, 1990). However, in some instances ethyl carbamate, which can exhibit carcinogenic activity in laboratory animals, may be present in yoghurt as a result of fermentation and/or the conversion reaction of diethyl pyrocarbonate to ethyl carbamate and levels reported ranged between <0.1 and 4.3 µg kg⁻¹ (Canas *et al.*, 1989; Hasegawa *et al.*, 1990; Sen *et al.*, 1993). Such levels of ethyl carbamate in yoghurt do not constitute any health risk to consumers.

Since all these types of preservative may be obtained in a powder form, they are added to the yoghurt milk with the rest of the dry ingredients; heat treatment of the milk does not affect their stability. However, in order to obtain maximum benefit from the preservative, the yoghurt must be of good quality and hence it is arguable whether its use is ever really justified.

2.7.3 Minerals, vitamins and/or fatty acids

2.7.3.1 Fluoridisation

Assali and White (1985) investigated fluoridisation (i.e. $4\mu\text{g g}^{-1}$) of yoghurt milk and no significant differences from the control were observed. Frank and Christen (1985) reported that the growth of lactic acid bacteria was not significantly affected in milk supplemented with Na-fluoride. This approach means that dairy products including yoghurt could be used as vehicles to provide children with additional fluoride in areas where water fluoridation is not practical. However, yoghurt producers would be well advised to note that the whole subject of fluoridation is extremely controversial. Thus, while few people object to the topical application of fluoride in toothpaste, the ingestion of fluoride in drinking water or in a food like yoghurt can, in some circumstances, be detrimental to health. For example, severe discoloration of teeth and damage to skeletal bones have been reported even with fluoride intakes within permitted guidelines and, given the healthy image of yoghurt, it would be most undesirable if the public perception of the product was damaged by a trendy gimmick.

2.7.3.2 Fatty acids

Caprioc or palmitic acids added at a rate of $0.01\text{ g }100\text{ g}^{-1}$ could not be detected as an off-flavour in pasteurised and homogenised milk, but caproic could be detected in dahi (Pantulu *et al.*, 1993). It has been noted also that modifying the feed of cows or the addition of soy oil to yoghurt milk did not affect the activity of the starter culture (Zbikowski *et al.*, 1982). The production of vegetable oil yoghurt is discussed in detail in Chapter 5.

2.7.3.3 Vitamins

Fortification of yoghurt by vitamins is targeted at children and has been marketed in some countries (Anon., 1983a). The stability of vitamins A and C in yoghurt was evaluated by Ilic and Ashoor (1988), Fiedlerova *et al.* (1993) and Noh *et al.* (1995). Both vitamins decreased during the storage period. This effect was minimised by using water-miscible beadlets of β -carotene (Parker *et al.*, 1992).

2.7.3.4 Low sodium

Low sodium yoghurt has become increasingly important for its nutritional properties and as a physiologically functional food. The milk base is processed in a cation exchange unit containing a strong acid resin (Nakazawa *et al.*, 1990). The quality of yoghurt made from low sodium milk was similar to the control and the reduced sodium level did not affect the activity of the starter culture. However, the sodium and potassium contents were reduced or increased, respectively, when compared with milk as follows: from 540 to $63\mu\text{g g}^{-1}$ and 1530 to $2360\mu\text{g g}^{-1}$, respectively. The

application of this approach to other cultured dairy products has been reviewed by Petik (1987).

2.7.3.5 Modified mineral content

Modification of the mineral content in yoghurt has been reported by many researchers. Some examples include the following processes on the milk base: (a) reducing the calcium content to 50% and enriching with magnesium up to 1 g l^{-1} (Pechery, 1985), (b) increasing the iron level in yoghurt by using iron tanks to ferment the milk (Coutsoucos and Colli, 1995; Batilde-Lima *et al.*, 1995) or adding iron to the milk base (Hekmat and McMahon, 1997), (c) increasing the calcium content in yoghurt using Ca-gluconate (Flinger *et al.*, 1988; Hansen and Flinger, 1996) and (d) the growth of *S. thermophilus* was reduced in calcium fortified milk and by using a higher inoculum, the rate of acid development was restored (Yousef and Rusli, 1995).

2.8 Homogenisation

Homogenisation means, quite literally, the provision of a homogeneous emulsion between two immiscible liquids, for example, oil/fat and water. The types of emulsion that may exist in dairy products are divided into two categories:

- Oil-in-water emulsion where the oil droplets are dispersed in the aqueous phase – the majority of homogenised dairy products fall into this category.
- Water-in-oil emulsion where the water droplets are dispersed in the oil phase – a typical example is butter.

Yoghurt milk is a typical oil-in-water emulsion and, as a result, the fat has a tendency to separate upon standing (especially in the fermentation tanks during the incubation period). In order to prevent this, the milk base is subjected to high speed mixing or homogenisation, that is, forcing the milk under high pressure through a small orifice or annulus. The overall relevance of this process to the manufacture of yoghurt is illustrated in Table 2.13.

However, these general effects are a reflection of the impact of homogenisation on specific milk constituents and in particular the effects described in the following Sections 2.8.1 and 2.8.2.

2.8.1 Effects on milk constituents

It is well established that the diameter of the fat globules in milk ranges from 1 to $10\mu\text{m}$, with an average around $3.5\mu\text{m}$. This variation in globule size is directly dependent on the same factors that influence the chemical composition of milk (i.e. breed of the cow, stage of lactation, age and health of the cow, type of feed, etc.). The effect of homogenisation is to reduce the average diameter of the fat globules to $<2\mu\text{m}$ (see Fig. 2.11), to prevent cluster formation and the tendency of the fat to rise to the surface and to decrease agglutination and elective bouyancy, due to the adsorption of casein micelles and submicelles.

The proteins in milk (casein and whey/serum protein) may undergo one or more of the following changes: (a) denaturation of some serum protein may occur, (b) casein/whey protein interactions may take place as result of denaturation of the

Table 2.13 Physical–chemical changes caused by homogenisation of milk used for yoghurt manufacture

Effect of homogenisation	Changes related to yoghurt
<i>Increase</i>	
Viscosity	Reduction in fat globule size and increased adsorption onto the casein micelle which increases the effective total volume of suspended matter.
Xanthin oxidase activity	Due to the disruption of the fat globule membrane which contains about half of the enzyme present in milk.
Colour (whiter)	Increase in number of fat globules which affects light reflectance and scattering.
Lipolysis	Increase in total fat surface area available to lipases; destruction of fat globule membrane which may enhance lipolysis by the starter culture.
Proper mixing	Especially if milk is fortified with milk powder.
Phospholipids in skimmed milk	As a result of the physical action, more fat globule membrane material is transferred to skimmed milk.
Foaming	As a result of increased phospholipids in the skimmed milk phase, pumping of yoghurt milk can cause foaming in the incubation tanks.
<i>Decrease</i>	
Fat globule size	Prevents ‘cream line’ formation in yoghurt, especially during incubation.
Oxidised flavour	Due to the migration of phospholipids to skimmed milk phase and formation of sulphhydryl compounds which act as antioxidants; possibly through denaturation of whey proteins causing exposure of hidden SH groups.
Protein stability	Changes in protein–protein interaction as a result of some denaturation and shift in salt balance.
Agglutination and effective buoyancy	Decrease in fat clustering due to adsorption of casein micelles and submicelles to fat globules.
Casein in skim phase	Partial transfer to casein from skimmed milk to form a new membrane around newly formed small fat globules (see Fig. 2.11).
Syneresis	Increase in hydrophilicity and water-binding capacity due to casein fat globule membrane interaction and other protein–protein interactions.

Adapted from Brunner (1974), Mulder and Walstra (1974), Harper (1976).

After Tamime and Deeth (1980). Reprinted with permission of *Journal of Food Protection*.

latter type of protein and/or a shift in the salt balance and (c) production of sulphhydryl compounds from denatured whey proteins may be observed. However, the effects and/or changes on the miscellaneous milk constituents are documented in Table 2.13, and these desirable effects of homogenisation can only be achieved if certain processing conditions are observed, namely:

- correct level of fat in the process mix
- correct homogenisation pressure
- correct temperature of homogenisation.

Before processing any type of mammalian full fat milk, there are no interactions between the major milk components, that is, the proteins (β -lactoglobulin (β -Lg),

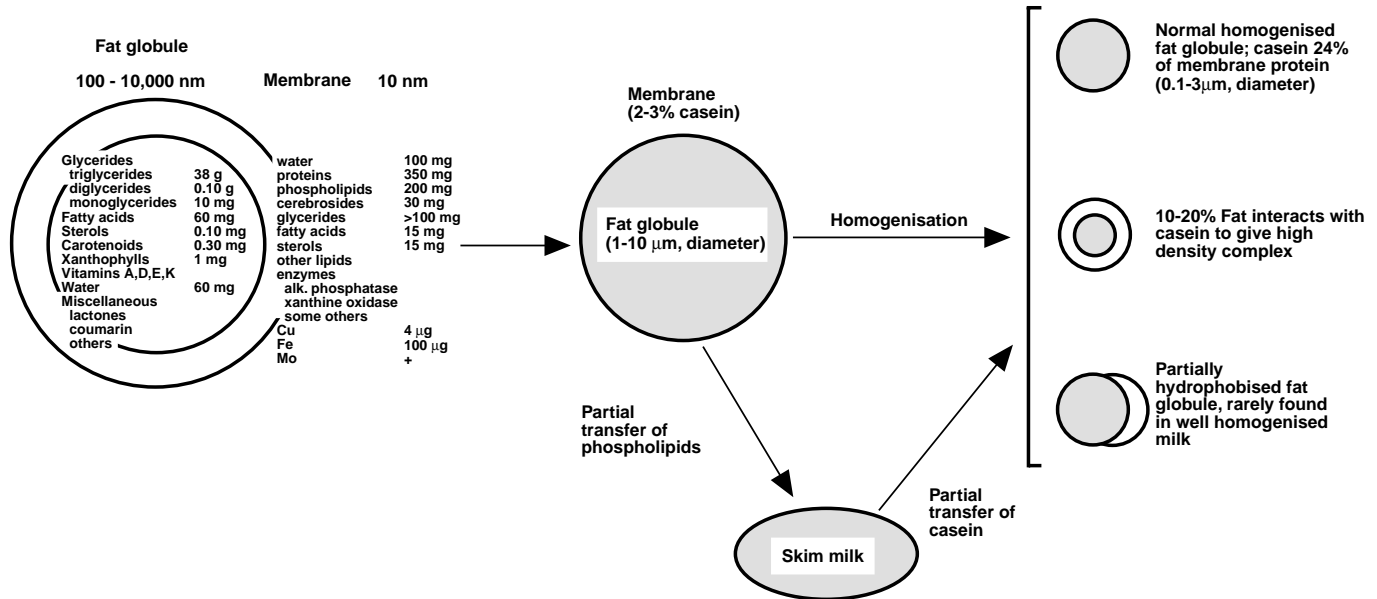


Fig. 2.11 Fat globule structure, composition and schematic representation of the effect of homogenisation on size
 $1 \text{ mm} = 10^3 \mu\text{m} = 10^6 \text{ nm} = 10^7 \text{ \AA}$.

Adapted from Mulder and Walstra (1974), Harper (1976) and Harper and Sieberling (1976).

α -lactalbumin (α -La) and the caseins), the fat and lactose (Walstra and Jenness, 1984). The fat constituent in raw milk is encapsulated within a membrane made of protein, lipids and phospholipids (Mulder and Walstra, 1974). Heat-induced and high pressure-induced processes cause chemical and physical changes in the milk fat globules. The chemical changes involve the fatty acids residue, but the effect of homogenisation and heating results in complex interactions between the milk components. Mulder and Walstra (1974), Dalglish and Sharma (1993), Sharma and Dalglish (1994), McCrae and Muir (1991), McCrae (1994), McCrae *et al.* (1994), Tomas *et al.* (1994), van Boekel and Walstra (1995), Dalglish *et al.* (1996) and Sharma *et al.* (1996) have reviewed the effect of these physical changes on the quality of many dairy products and possible changes applicable to homogenisation before heat treatment are:

- Break up of the fat globules (1–10 μ m) to give particle sizes in the sub-micron range.
- Some of the casein micelles break up and bind with newly formed fat globules to stabilise them.
- Serum proteins have a relatively minor role, but some may interact with the fat globules in the absence of heating.
- The fat particles in the homogenised milk have a different structure from the native fat globule, and hence they have different properties with respect to coagulation of milk by enzymes or heat; the enzymes are relevant in cheesemaking.

However, when the milk is heated, the induced interactions are more significant (for details refer to Section 2.9) and can be summarised as follows:

- Denaturation of α -La and β -Lg takes place, with subsequent interactions, principally of the β -Lg whey/serum protein.
- β -Lg becomes more reactive after denaturation due to the presence of unpaired sulphhydryl (SH) groups.
- Possible reactions of denatured β -Lg include interaction with other β -Lg, interaction with κ -casein on the surface of casein micelles and interaction with fat globule membrane which results in an approximate doubling of the amount of fat-bound protein.

Thus, heating of homogenised milk $\geq 70^\circ\text{C}$ results in new structures being formed, mainly the denatured whey/serum proteins which may undergo further reactions such as:

- interaction with other denatured β -Lg to form a gel;
- interaction with κ -casein on the surface of micelles in suspension;
- interaction with κ -casein adsorbed onto the fat globules;
- interaction with residual fat globule membrane;
- adsorption onto the fat globule surface, i.e. displacing the adsorbed caseins.

2.8.2 Aspects of processing

The use of single stage or double stage homogenisation is only critical in products containing high levels of fat (e.g. cream) and since the fat in cream has a tendency to recluster, double stage homogenisation is recommended. However, yoghurt milk is usually processed through a single stage homogeniser at around 65–70°C and at

pressures ranging between 15 to 20 MPa. Pressures up to 30 MPa have been reported, but in practice they are not widely used. Kebary and Morris (1989) studied the effect of homogenisation (i.e. two stage up to 27.6 + 3.5 MPa) on fat clustering and the distribution of fat globules in recombined milks, and these effects increased as the homogenisation pressure and fat content increased. According to the review by Tamime and Marshall (1997), the effect of homogenisation of the yoghurt milk and subsequently the quality of the manufactured product are that: (a) the fat surface area is increased, the size of the globule is decreased and the composition of the membrane is different, (b) in part, the fat surface is coated with surface-active materials, mainly proteins, (c) the turbulent effect of homogenisation favours the adsorption of casein micelles over serum proteins (c. 5%), so covering 25% of the surface area of the fat globule, (d) in recombination (i.e. the milk fat is homogenised into the skimmed milk) the resulting fat globule membrane consists only of serum protein, (e) the homogenised fat globules act as large casein micelles (i.e. because the membrane consists mainly of caseins) which increase the effective casein concentration, and hence, participate in casein reactions such as acid precipitation, (f) the increased number of small fat globules enhances the ability of the milk to reflect light and, as a result, the fermented milk appears whiter and (g) the risk of syneresis (i.e. separation of free whey onto the surface of set fermented milk) is reduced, and the firmness of the end product is increased giving it a better mouth-feel.

In some instances, homogenisation of the yoghurt milk takes place after heat treatment of the milk base, but this approach carries with it the risk of contamination unless high standards of hygiene are observed and/or an aseptic homogeniser is used. Kulkarni *et al.* (1990c, d) reported that when using 30% WPC (about 25 g TS/100 g⁻¹) for the production of cream yoghurt (10 g fat/100 g⁻¹), the following process could be recommended: (a) improving the product by adding 2% SMP, (b) heating the milk to 95°C for 22 s to achieve 70% denaturation of β -Lg and (c) finally homogenising at 75°C and 20 MPa pressure. However, in separate reports, Plock *et al.* (1992) and Huss and Kessler (1991) evaluated different processing parameters (e.g. casein-to-whey ratio, one-stage homogenisation at pressures ranging between 5 and 30 MPa or homogenising the milk base many times before or after the heat treatment stage at 95°C for 80 s), and they concluded that maximum gel consistency and water-holding capacity of yoghurt was achieved with homogenisation at ≤ 25 MPa pressure after heat treatment. Whether or not the homogenisation of milk after heating is beneficial to product quality has, of course, to be verified further. Thus, when a batch of cow's milk with 16% TS (vacuum evaporation) was divided into two and homogenised either before or after heating, the textures (set yoghurts) and viscosities (stirred yoghurts) were identical (Robinson, unpublished data). Perhaps the method of fortification is critical and/or the precise temperature regime, but clearly the situation is in need of further clarification. Ozer (unpublished data) observed a similar pattern for both cow's and sheep's milks, but homogenisation after heat treatment did improve the textural properties of goat's milk yoghurt. Nevertheless, the improved viscosity of yoghurt that is reported to follow homogenisation of the milk is due primarily to:

- a change in the water-holding capacity of the milk proteins which tends to reduce syneresis (Grigorov, 1966a; Kessler and Kammerlehner, 1982; Kneifel and Seiler, 1993);

- the increased amount of milk fat globule membrane material, i.e. phospholipids and proteins in the skim phase, which may also improve the water-holding capacity of the coagulum (Samuelsson and Christiansen, 1978);
- the rate of acidification of the milk which was increased by increasing the pressure of homogenisation (i.e. 0–15 MPa) (Volkova and Radulov, 1986).
- the curd tension of the bio-yoghurt which was influenced significantly ($P \leq 0.01$) by the level of SNF ($18\text{ g } 100\text{ g}^{-1}$) and two-stage homogenisation (pressures of 14.6 and 3.5 MPa), while the fat content ($4.5\text{ g } 100\text{ g}^{-1}$) also affected the characteristics of the curd ($P \leq 0.05$); however, holding times of the heated milk at 90°C had no effect (Asgar and Thompkinson, 1994).
- improvements in the physical properties of yoghurt which were achieved by fortification of the milk using UF method, heating of the milk between 100 and 120°C for 4 or 16s and two-stage homogenisation (14.2 and 3.5 MPa, respectively) at 55°C after heating the milk (Savello and Dargan, 1995).
- yoghurt milk homogenised at 0, 10.3 and 34.5 MPa pressures which showed differences only in syneresis and water-holding capacity (Schmidt and Bledsoe, 1995).
- replacement of the milk fat with vegetable oils or fat substitutes which affects the rheological, sensory characters and the microstructure of yoghurt (for more detail refer to Chapter 5).

In addition, the processing conditions (temperature and pressure) employed during the homogenisation of the milk base can affect the extent of any changes (Misra, 1992). Storgards (1964) produced an increase in the viscosity of sour milk by progressively increasing the pressure (i.e. from about 5 to 30 MPa) of homogenisation without heating the milk; a similar trend was also reported for milk subjected to heat treatment (Fig. 2.12). This effect was previously reported by Galesloot (1958) and a summary of his results is presented in Table 2.14. Abrahamsen and Holmen

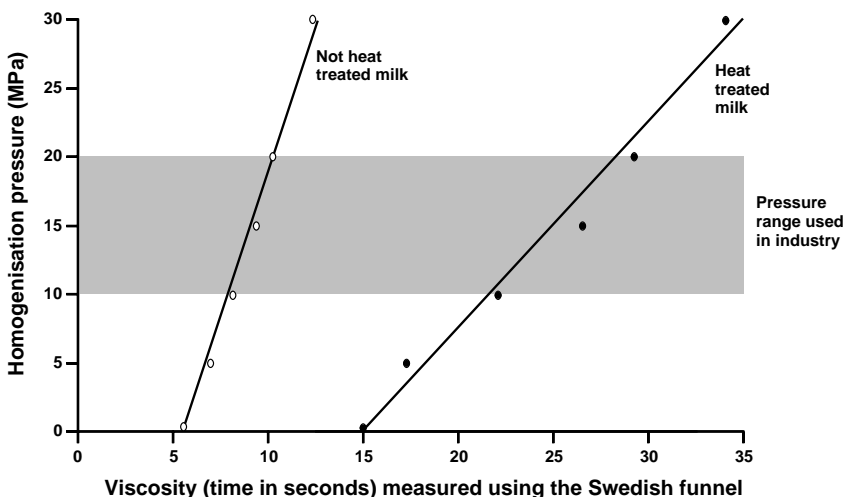


Fig. 2.12 Effect of different homogenisation pressures on the consistency/viscosity of sour milk

Data compiled from Storgards (1964).

Table 2.14 Effect of homogenisation and heat treatment on the consistency/viscosity of yoghurt

Measurement of consistency/viscosity of yoghurt	Heat treatment of milk for 30 min at							
	70°C		78°C		86°C		95°C	
	A	B	A	B	A	B	A	B
Falling sphere ^a (depth, cm)	3.0	>15.0	1.5	10.5	1.2	6.0	1.2	2.7
Posthumus funnel ^b (time, s)	9.0	5.0	14.0	7.5	17.0	8.5	18.7	9.0

A, Homogenised milk; B, non-homogenised milk.

^a Falling sphere: the deeper the sphere sinks into the yoghurt, the thinner is the product. ^b Posthumus funnel: the longer the time required for the yoghurt to pass through the funnel, the more viscous is the product.

Data compiled from Galesloot (1958).

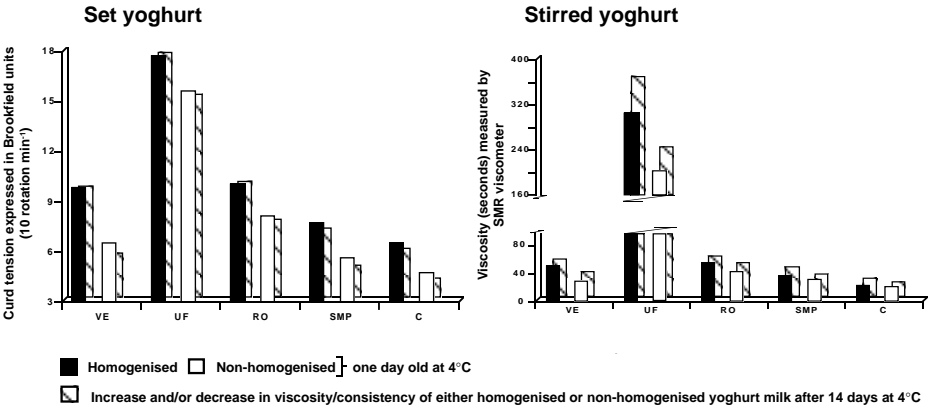


Fig. 2.13 Viscosity/consistency of goat's milk yoghurt (homogenised and non-homogenised) concentrated by different methods and stored for 1–14 days at 4°C

VE: Vacuum evaporation, UF: ultrafiltration, RO: reverse osmosis, SMP: skimmed milk powder, C: control.

Data compiled from Abrahamsen and Holmen (1981).

(1981) studied the quality of goat's milk yoghurt manufactured from homogenised and non-homogenised milks concentrated by different methods and they concluded that: (a) homogenisation of goat's milk was essential for yoghurt production (Fig. 2.13), (b) a reduction in the consistency of set yoghurt was reported after 14 days' storage and the best results were obtained when the goat's milk was concentrated using ultrafiltration and (c) goat's milk yoghurt had a lower viscosity than yoghurt made with cow's milk due to the low protein content of the goat's milk (Abrahamsen and Holmen, 1980, 1981). A similar observation was reported by Muir and Tamime (1993) on the firmness of sheep's milk yoghurt made from homogenised and non-homogenised milks without fortification. For more details refer to Chapter 5.

2.9 Heat treatment

Although the application of heat, that is, boiling of milk, has long been practised during the manufacture of yoghurt as a method of increasing the concentration of milk solids in the milk base, in the present context the effects of heat treatment can be broadly summarised as:

- destruction and/or elimination of pathogens and other undesirable micro-organisms;
- production of factors stimulatory/inhibitory to the yoghurt starter cultures;
- changes in the physicochemical properties of the milk constituents which are relevant in yoghurt making.

In commercial practice, heating of milk is the most widely used unit operation in the manufacture of a wide range of dairy products. The time/temperature combinations applied range from $\leq 65^{\circ}\text{C}$ (thermisation) for a few seconds to 150°C for a few seconds for ultra high temperature (UHT) sterilisation. Milk for the manufacture of yoghurt is heated at different temperatures and the reported treatments, including the processing of liquid milk, are illustrated in Table 2.15. The choice of any one particular time–temperature combination is based on a number of factors, but assuming that there are no limitations imposed by the plant itself, those mentioned above tend to be the dominant considerations.

Table 2.15 Some time/temperature combinations used during the processing of liquid milk and yoghurt milk base

Time	Temperature ($^{\circ}\text{C}$)	Process	Comments
Few s	≤ 65	Thermisation	Main purpose is to kill psychotropic bacteria; it causes no other irreversible changes.
30 min	65	Batch pasteurisation	Destruction of almost all pathogenic organisms present in milk, but not all vegetative cells of micro-organisms are killed; inactivation of some enzymes; flavour and whey proteins remain unchanged.
15 s	72	Pasteurisation	
4–20 s	85	High pasteurisation	Destruction of all vegetative cells, but not bacterial spores; most enzymes are destroyed, but not milk and bacterial proteinases or bacterial lipases; denaturation of whey proteins.
30 min ^a	85		
5 min ^a	90–95		
40–20 min	110–120	In-container sterilisation and autoclaving	Destruction of all micro-organisms and spores; some UHT treatment may not suffice to inactivate all enzymes; chemical changes, colour and flavour of milks are affected.
20–2 s	135–150	UHT	

^a Heat treatments that are widely used in the yoghurt industry.

Table 2.16 Chemical and physical effects of heat treatment of milk and their relevance in yoghurt manufacture

Milk constituent	Heat-induced changes	Relevance in yoghurt manufacture	Consequences for yoghurt
<i>Nitrogenous</i>			
Whey proteins	Denaturation and aggregation, inactivation of immunoglobulins Active –SH group production	Almost complete Maximum at 90°C/10 min	Destruction of lactenins, reduction in creaming ability Cooked flavour, lowering of Eh, formation of antioxidant properties
	α -La and β -Lg interaction	Occurs before and/or interaction with κ -casein	Contributes to gel stability
	β -Lg and κ -casein interaction	Very significant	Minimises syneresis, increases micelle size, stabilises gel
Casein	Partial hydrolysis, release of glycopeptide from κ -casein Dephosphorylation	Of limited significance Very little	Slight increase in free amino acids and peptides Slight redistribution of phosphorus
	Aggregation, disaggregation, interchain cross-linking, e.g. by isopeptide bonding	Occurs especially with smaller micelles	Increase in micelle size and formation of protein network
Enzymes	Inactivation	Destruction of lipases and proteases from milk and bacteria	Minimises rancid and bitter off-flavours
Other	Decomposition of amino acids to flavour compounds	Significant effect	Contributes to flavour
	Amino acid–lactose interaction, Maillard reaction, Schiff's base formation, reduction in available lysine	Occurs to only small degree, e.g. lysine loss <i>c.</i> 0.3%	Slight decrease in nutritive value, significant where yoghurt fortified with high-heat powders and concentrates
	Amino acid–amino acid interaction, e.g. formation of lysine–alanine	Occurs to a limited degree	Minimal

Milk constituent	Heat-induced changes	Relevance in yoghurt manufacture	Consequences for yoghurt
<i>Carbohydrates</i>			
Lactose	Decomposition to form organic acids, furfural and hydroxymethylfurfural Reaction with amino acids (see above)	Occurs to small extent	Reduces pH and Eh, produces formic acid and affects growth of starter cultures, contributes to yoghurt flavour
Other	Decrease in sialic acid and hexosamines, increase in hexoses	Occurs at 85°C for 10min	Unknown
<i>Miscellaneous</i>			
Fat	Formation of lactones, methyl ketones and other volatile ketones Hydrolysis	Occurs to small degree Insignificant	Contributes to flavour Insignificant
Vitamins	Destruction of some water-soluble vitamins	C, B ₁ , B ₆ , B ₁₂ , folic acid, reduced	Reduction in nutritive value
Minerals	Redistribution of Ca, P, Mg between soluble and colloidal forms	Significant effect, modifies surface structure of casein micelle	Reduces pH, affects curd particles, decreases coagulation time
Micro-organism	Destruction	Elimination of pathogens and other organisms	Ensures public safety and minimises quality defects
Gases	Reduction in level of dissolved oxygen, nitrogen and carbon dioxide	Produces micro-aerophilic environment for starter culture	Ensures public safety and minimises quality defects

Compiled from Tamime and Deeth (1980). Reprinted with permission from *Journal of Food Protection*.

Thermal treatment of milk has been extensively studied in relation to many aspects such as heat-induced changes to milk constituents, changes in nutritional properties of milk, inactivation of enzymes (indigenous and/or of bacterial origin) and functional properties of dairy products (e.g. heat stability of UHT milk, evaporated milk and milk powder) (see Table 2.16); the reviews by Fox (1989, 1991, 1995) are recommended for further reading. During the manufacture of yoghurt, milk is heated at $>70^{\circ}\text{C}$ and the physical and chemical changes that can occur in the milk base are complex and multifunctional. The impact of thermal processing relevant to the functional properties of yoghurt is summarised below.

2.9.1 Destruction of micro-organisms/pathogens

The heat treatment of the yoghurt milk at $85\text{--}95^{\circ}\text{C}$ (Table 2.15) is sufficient to kill the majority, if not all, of the vegetative cells of micro-organisms associated with raw milk (Gilmour and Rowe, 1990), but spore formers and some heat-stable enzymes will remain. This reduced competition ensures that the heated milk will provide a good growth medium for the yoghurt starter culture, but nevertheless, the bacteriological quality of the raw milk and any dry ingredients used in the milk base is of great importance.

Thus, a high level of psychotrophic bacteria can break down both β -casein and α -casein (DeBeukellar *et al.*, 1977) and the fat constituents in milk, and while the degradation of casein can lead to the formation of a weak coagulum and subsequent whey separation, hydrolytic rancidity can give rise to serious off-flavours (Cousin, 1977; Cousin and Marth, 1977a, b). It is also important that the enzymes (peptide hydrolases and lipases) of some *Pseudomonas* spp. are heat stable, and extremely high heat treatments (150°C) are required to inactivate them (Mayerhofer *et al.*, 1973; Kishonti, 1975; Adams *et al.*, 1975; Barach *et al.*, 1976, 1978; Hedlung, 1976; Adams and Brawley, 1981; Fairbairn and Law, 1986; Stead, 1986; McKellar, 1989; Driessen, 1989; Stepaniak and Sørhang, 1995).

Indigenous enzymes (c. 60) have been identified in raw milk and some of these enzymes are heat labile, while others can survive the UHT treatment of milk. The role of these enzymes in dairying has been critically reviewed by Fox (1991) and Farkye and Imafidon (1995). The activities of milk enzymes have been useful indicators of diseases or physiological changes in the udder of the mammal, of processing conditions applied to milk and of factors influencing the flavour and quality of dairy products. Fortunately, the survival of these enzymes has not been identified as a significant problem in the yoghurt industry (Cogan, 1977).

2.9.2 Production of stimulatory/inhibitory factors

The heating of milk can result in the release of certain factors that can either stimulate or inhibit the activity of lactic starter cultures. The work of Greene and Jezeski (1957a–c) summarises the overall events:

- stimulation of the starter culture in milk heated between 62°C for 30 min and 72°C for 40 min;
- inhibition of the starter culture in milk heated between 72°C for 45 min and 82°C for 10–120 min or 90°C for 1–45 min;
- stimulation of the starter culture in milk heated between 90°C for 60–180 min and autoclaving at 120°C for 15–30 min;

- inhibition of the starter culture in milk heated by autoclaving (120°C) for more than 30 min.

The apparent stimulation/inhibition/stimulation/inhibition cycle was due to changes in the serum or whey proteins and the above cycle could be simulated by the addition of denatured whey proteins or cysteine hydrochloride. The transition from one cycle to another, in response to different heat treatments, could well reflect the release of denatured nitrogenous compound(s) (e.g. at concentrations of 0.15–0.20 mg ml⁻¹) or from 10 to 20 µg ml⁻¹ of cysteine, since when cysteine was added artificially, it augmented the sulphhydryl groups made available by heating; cysteine became stimulatory in raw and low heated milks, but in highly heated milks the same concentration became inhibitory. Taking this idea further, the same workers offered the following explanation for the stimulation/inhibition cycles:

- The initial stimulation was attributed to the multitude of factors listed in Table 2.16.
- The release of cysteine, glutathione or thioglycolate and the expulsion of oxygen resulted in the stimulatory effect.
- The inhibition was due to an excess concentration of cysteine in the milk, accompanied by an increase in toxic volatile sulphides.
- The second cycle of stimulation was due to a reduction in the level of toxic sulphides as a result of further heating, or perhaps the formation of formic acid.

Consequently, Greene and Jezeski (1957a–c) recommended the use of high-heat powders, but such observations may not be applicable at the present time in view of (a) developments in powder manufacture technology and (b) improved selection of starter culture strains. However, refer to Section 2.4.2 for further detail.

Dutta *et al.* (1973) investigated the effect of different heat treatments on acid and flavour production by various single strains of lactic acid bacteria, including *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* and a summary of their work is given in Table 2.17. Overall the degree of heating had a rather variable effect on the activity of the yoghurt starter cultures, but the reasons for this behaviour were not discussed; however, it is most likely that changes to certain milk constituents (Table 2.16) promoted the observed variation in the activity of the starter cultures.

Table 2.17 Effect of heat treatment of milk on the activity of yoghurt starter cultures

Value	<i>S. thermophilus</i>			<i>L. delbrueckii</i> subsp. <i>bulgaricus</i>		
	63°C/ 30 min	85°C/ 30 min	Steaming/ 30 min	63°C/ 30 min	85°C/ 30 min	Steaming/ 30 min
Titrateable acidity ^a	1.00	0.85	0.66	1.60	1.70	1.62
Volatile acidity ^b	9.00	9.00	7.00	40.00	34.50	31.00
Diacetyl (µg g ⁻¹) ^c	13.00	12.00	6.00	12.00	13.00	0.00
Proteolytic activity ^d	0.34	0.25	0.18	0.25	0.18	0.09

^a Lactic acid (%); ^b ml of 0.01 N NaOH 50 g⁻¹ of curd; ^c Level of diacetyl is abnormally high (see Chapter 7); ^d mg of tyrosine liberated g⁻¹ of curd.

After Dutta *et al.* (1973). Reprinted with permission of *Milchwissenschaft*.

2.9.3 Changes in the physicochemical properties of milk

Fresh liquid milk is composed of around 87g100g⁻¹ water and 13g100g⁻¹ total solids, and the composition of yoghurt milk (after being standardised and/or fortified) is slightly altered to 84–86g100g⁻¹ water and 14–16g100g⁻¹ total solids. It may appear from such data that milk is simple in its composition, but on the contrary, milk has a very complex structure (see Fig. 2.2), even though its constituents are mainly water, carbohydrates, fat, proteins and minerals. These different components appear to be dispersed between two colloidal systems, that is, the fat globules and their membranes (Fig. 2.11), and the casein micelle complexes. In general, both colloidal systems are heat stable, and the effects of heat treatment on them and the relevance of these to yoghurt manufacture are summarised in Table 2.16. It is apparent from this data that yoghurt milk undergoes several changes during the heat treatment.

2.9.3.1 Effect on the proteins

Some detailed studies of the proteins in cow's milk have been reported by Cheeseman (1975), Whitney *et al.* (1976), Eigel *et al.* (1984), Banks and Dalgleish (1990), Walstra (1990), Dalgleish (1990a), Farrell *et al.* (1990), Creamer (1991), Fox (1992), Jakob (1994), Fox and Flynn (1994) and Holt and Horne (1996). In addition, the interactions of milk components including the basic chemistry and the action of milk proteins in different food systems have been reviewed by de Wit (1990a), Jost *et al.* (1990), Creamer *et al.* (1994) and Pearce (1994). The various constituents that go to make up the total protein content of milk are:

Casein		76–88% of total protein
α _{s1} -	45–55	} % of fraction indicated
β-	25–35	
κ-	8–15	
γ-	3–7	
Whey proteins		15–22% of total protein
Serum albumin	0.7–1.3	} % of fraction indicated
β-lactoglobulin	7–12	
α-lactalbumin	2–5	
Immunoglobulin	1.9–3.3	
Proteose/peptones		2–6% of total protein

The casein in milk constitutes the major group of bovine proteins which play an important role during the manufacture of certain dairy products, for example yoghurt and cheese. The structures of these proteins, based on some models that have been proposed, suggest that the caseins exist as micelles or aggregates of sub-micelles which are basically formed from α_s- and β-casein stabilised by κ-casein in association with calcium and calcium phosphates (Banks and Dalgleish, 1990).

The other types of protein (i.e. serum or whey proteins) appear to be in solution, and they have a more defined, compact, globular shape than the caseins. This structure is due to the formation of disulphide bonds (as a result of the cysteinyl residues present), the lack of phosphate groups and the fact that they do not react with calcium or aggregate together in the native state (Banks and Dalgleish, 1990). The functional properties of the whey proteins become more apparent after heating the milk, since at temperatures above 80°C, they are denatured and react/bind with κ-casein to form a more stable micelle.

Table 2.18 Changes (%) in nitrogenous fractions of milk from different species after heating

Nitrogen fraction	Cow			Goat			Sheep		
	63°C/ 30 min	80°C/ 10 min	120°C/ 15 min	63°C/ 30 min	80°C/ 10 min	120°C/ 15 min	63°C/ 30 min	80°C/ 10 min	120°C/ 15 min
Casein	+0.60	+14.95	+18.77	0	+24.43	+25.31	+3.98	+26.35	+2.35
Non-casein	-1.61	-45.63	-57.77	0	-35.90	-41.81	-10.50	-68.06	-76.05
Soluble protein	-2.23	-62.40	-89.38	0	-58.08	-70.58	-15.07	-79.39	-93.96
β -Lactoglobulin	-1.80	-59.56	-94.30	0	NR	-100.00	-34.65	-74.25	-100.00
Non-protein	0	0	+25.00	0	+4.76	+4.76	+11.36	+15.00	+15.00

NR: not reported.

Adapted from Ramos (1978).

A good example of this effect is observed when milk is heated to 90°C (forewarmed) for a period of time that ensures complete reaction between the different types of proteins, since it can then be heated to 120–140°C to give a stable end product (e.g. UHT milk). Comparative data for the effect of heat on the milk proteins (including caseins) of different species are illustrated in Table 2.18.

Caseins, as mentioned elsewhere, are heat stable when compared with whey proteins. Thus, β -Lg and α -La are denatured at the temperatures employed for the processing of the milk base (Dannenberg and Kessler, 1988a, b; de Wit, 1990b; Pearce, 1994; Law, 1995), and while β -Lg reacts with other milk components when denatured, β -La undergoes heat-induced interactions only after severe heat treatment (Dalgleish and Sharma, 1993; Sharma and Dalgleish, 1994). The possible interactions are:

- The association of small aggregates of denatured β -Lg molecules to form larger aggregates (Xiong *et al.*, 1993), or as a function of pH and temperature (MacLeod *et al.*, 1995).
- The interactions between β -Lg and κ -casein as a result of heating the milk involve hydrophobic interactions of exposed SH groups (Haque and Kinsella, 1988; Noh and Richardson, 1989; Dalgleish, 1990b).
- During the heating of milk at $\leq 90^\circ\text{C}$, the interactions of β -Lg and α -La with the casein micelles have similar kinetics but when heating the milk in UHT system, α -La reacts more slowly than β -Lg due to the rate of heat transfer (Corredig and Dalgleish, 1996a, b).
- Heat treatment may extensively modify one of the fat globule membrane proteins, c. 49 kDa (Kim and Jimenez-Flores, 1995) and, as a consequence, the interactions between denatured whey proteins and the fat globule membrane proteins may not be explained solely by —SS linkages.
- The attachment of κ -casein onto the surface of the fat globule membranes results in losses of triacylglycerols and changes in lipid content upon heating milk at 80°C for 20 min (Houlihan *et al.*, 1992a, b; Singh, 1993; van Boekel and Walstra, 1995).
- Interaction of β -Lg with the homogenised milk fat globule surface may displace the adsorbed micellar caseins (Xiong and Kinsella, 1991a, b; Dalgleish and Sharma, 1993; Sharma and Dalgleish, 1993, 1994).

Table 2.19 Effect of heat treatment on the coagulation process during the manufacture of yoghurt

Item	Heat treatment of milk					
	85°C	85°C/30 min	90°C	90°C/30 min	95°C	95°C/30 min
Coagulation time (hour)	2.43	2.01	2.34	2.04	2.29	2.04
Acidity at coagulation (% lactic acid)	0.63	0.49	0.63	0.50	0.63	0.50
pH at coagulation	4.70	5.16	4.78	5.12	4.80	5.08

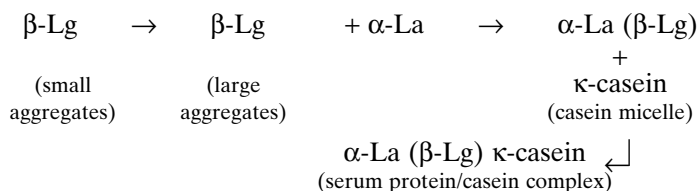
Adapted from Grigorov (1966b).

- Binding of colloidal calcium phosphates and other ions by the caseins; this shift in the ionic constituents is not critical in acid gel formation (Schmidt and Poll, 1986; Aoki *et al.* 1987a, b, 1988, 1990; Wahlgren *et al.*, 1990; Holt, 1995; Zhang and Aoki, 1995).
- Aggregation of casein micelles into larger particles and also dissociation of casein micelles to form soluble caseins at 100°C or above (Singh, 1993; Law, 1996).

It is evident that heat-induced changes of the proteins in milk, and subsequently acid development (see Section 2.10.3 on gel formation), can affect other properties. For example, the optimum hydrophilic properties of the proteins are obtained when the milk is heated to 85°C for 30 min (Grigorov, 1966a–c). The effect of different heat treatments on the coagulation of cow’s milk is shown in Table 2.19. The observed improvements in the rate of gel formation are possibly due to interactions between β -Lg and casein, since heating milk at 80°C for 30 min denatures more than 90% of the β -Lg compared with only 60% of the α -La (Larson and Roller, 1955). Maximal hydration of the protein, according to Grigorov (1966c), occurs when milk is heated at 85°C and decreases gradually as the temperature rises; this view is shared by many researchers, including Prodanski (1967) and Iyengar *et al.* (1967). This decrease in the hydrophilic properties of the casein/ β -Lg complex can adversely affect the quality of the yoghurt, possibly increasing the tendency to syneresis, and hence, ignoring other considerations, the heat treatment of milk intended for the production of yoghurt should be between 85°C and 95°C.

The effect of heat on the proteins, according to Parry (1974), is a two-stage process: first, the structure is altered causing denaturation, and second, aggregation takes place followed by coagulation, depending on the level and duration of heating; β -Lg undergoes such a process when the —SH groups are reactivated as a result of heating (Walstra and Jenness, 1984). The aggregates are of two sizes, depending upon which reactive groups are involved, that is, small aggregates of β -Lg (3.7S) with interlinking —SH groups, and larger aggregates of β -Lg (29S) in which the formation of —SS bonds may be important (some early references have been cited by Sawyer, 1969; McKenzie, 1971; Lyster, 1979).

In the 1970s, the information published on the heat denaturation of β -Lg recognised the interaction between β -Lg and κ -casein, but reports by Elfagm and Wheelock (1977, 1978a, b) suggest α -La is also involved. In brief the interaction may be as follows:



However, more recently Mottar *et al.* (1989) have proposed a slightly modified model in which the denatured β -Lg becomes associated with κ -casein of the casein micelles (i.e. phase 1). This results in the formation of appendages to the casein micelles which are irregular in structure and the surface becomes highly hydrophobic. When α -La starts to denature (i.e. phase 2), it interacts with β -Lg and fills the structural gaps formed in phase 1. The amount of α -La present to the micellar surface is dependent on the heating process and its intensity. This results in a smoother surface with decreased hydrophobicity and an increased water-holding capacity of the protein matrix (see also Hill, 1989). In a recent study, Calvo *et al.* (1993) concluded that thermally induced aggregation of α -La was dependent upon the concentration of free —SH groups present in other whey proteins; this appeared to function by inducing cleavage of the intramolecular —SS bonds in α -La leading to aggregation.

2.9.3.2 Effect on rate of denaturation of whey proteins

Law (1995, 1996) has quantitatively studied the relative rates of irreversible denaturation of whey proteins (i.e. immunoglobulins, serum albumin/lactoferrin, β -Lg and α -La) of different mammalian milks on heating at 70–90°C. The reported results suggest that (a) the concentrations of the individual whey proteins in cow's, goat's and sheep's milk are different, for example, the total whey protein contents were 0.65, 0.61 and 1.1 g 100 ml⁻¹, respectively, (b) upon heating, the order of denaturation of the milk of the three species of mammals was immunoglobulins > serum albumin/lactoferrin > β -Lg > α -La, and (c) at 90°C, the order of ease of denaturation of whey proteins was sheep > goat > cow. Other studies of the effect of heating on proteins have been reported by Law and Tziboula (1992, 1993), Law *et al.* (1993, 1994), Brown *et al.* (1995) and Tziboula (1997). The addition of a thermolabile variant of β -LgA to raw milk reduced syneresis of yoghurt when the milk base was processed at 70°C (Lee *et al.*, 1994; Batt *et al.*, 1994); this approach to yoghurt making could encourage future manipulation of the protein constituent(s) during their synthesis in the udder of the cow.

2.9.3.3 Effect on protein/fat interactions

Protein/fat interactions in recombined milks have been studied extensively by Singh and Creamer (1991) and Singh *et al.* (1993, 1996a). They concluded that these interactions are dependent on many factors, such as:

- Increasing the protein content in skimmed milk results in an increase in the protein load on the fat surface to reach a maximum of about 6 mg per m².
- Large fat globules have a lower protein load (i.e. mainly whey proteins).
- Altering the whey protein-to-casein ratio in the skimmed milk decreases the protein load adsorbed on the surface of the fat globules and can influence the composition of the protein layer.

- The extent of κ -casein dissociation increases when the SNF is increased from 10 to 20 g 100 g⁻¹ in recombined skimmed milk at pH 6.5–7.1 before heating, and/or when it is heated at 120°C for 2–11 min at pH 6.5 only.
- The rate of dissociation of κ -casein from the fat globule surface and the casein micelles was pH dependent.
- The protein load on the fat globule surface is decreased and the composition of the adsorbed protein is altered on disintegration of the casein micelles following the removal of colloidal calcium phosphate.

2.9.3.4 *Effects on other milk constituents*

It is evident that the component(s) in milk which are most dramatically modified by heat treatment at the temperatures practised in the yoghurt industry are the whey proteins, but other heat-induced changes can occur in milk and are of some significance. These include: (a) heating milk can affect the state of the milk salts, particularly calcium, phosphate, citrate and magnesium. Thus, these salts may exist in milk as soluble ions or in the colloidal phase as part of the casein micelle complex, and heating milk to 85°C for 30 min can change up to 16% of the soluble calcium into the colloidal phase (Kannan and Jenness, 1961). (b) Heating milk may reduce the amount of oxygen present, that is, lowering the redox potential, which encourages starter culture growth. (c) Undesirable flavours in milk are often removed by ordinary heat treatments, but severe heating can induce off-flavours, for example, the caramel flavour that results from the Maillard reaction between lactose and the amino groups of the proteins. (d) Vitamins in milk are subdivided into two main groups, the so-called fat-soluble vitamins (e.g. A, D, E and K) associated with the fat component of milk and the water-soluble vitamins (e.g. B group and C). The former vitamins are fairly heat stable, while vitamins B₆, B₁₂ and C are heat labile. Table 2.20 illustrates the percentage losses of the heat sensitive vitamins in milk during different heat treatments. The relatively high heat treatments used in yoghurt

Table 2.20 Typical values of vitamin losses (%) from milk during different heat treatments^a or processes

Vitamins	Pasteurisation		Sterilisation		Evaporated	Powders	
	Batch	Low	In-container ^b	UHT		Roller	Spray
Thiamine (B ₁)	10	<10	20–35	10	20–60	20–30	10–15
Riboflavin (B ₂)	N	N	<10	10	0	10–15	<10
Folic acid	0	0	40–50	15	–	–	–
Biotin	<10	<10	<10	<10	10–15	10–15	10–15
Pantothenic acid	N	N	<10	<10	<10	<10	<10
B ₆	10	0	60–90	<10	90	20	35
B ₁₂	20	10	40–50	10	60	30	20

^a For further details refer to Table 2.15. ^b Heat treatments used: 110°C for 15 min and 115°C for 30 min; higher losses resulted during the latter treatment.

N, Data not available. –, Negligible.

The heat liability of vitamins A(+/-), carotene (-), D(-), E(+/-), K(?) and C(++) are not listed.

After Chapman *et al.* (1957), Porter and Thompson, (1972), Kon (1972), Ford and Thompson (1981), Renner (1983, 1989), Walstra and Jenness (1984), Burton (1988), Schaafsma (1989), Andersson and Öste (1995).

manufacture may, therefore, cause significant decreases in some vitamins and the presence of dissolved oxygen greatly enhances the sensitivity of the heat-labile vitamins (Hartman and Dryden, 1974; Renner, 1983, 1989; Walstra and Jenness, 1984; Burton, 1988; Andersson and Öste, 1995; Sharma and Lal, 1995). (e) Although heating the yoghurt milk base may destroy some vitamins and adversely affect the nutritional properties of yoghurt, the digestibility of proteins in the intestinal tract may be improved in comparison with unheated milk proteins (Puhan, 1988). (f) As a result of heat-induced changes in the milk constituents during heat treatment, fouling or formation of deposits on the surfaces of the processing equipment will occur. Thus, the operational time of the heat exchangers will be shortened and more cleaning is required. Studies on the different types of fouling of heating surfaces and the role of milk constituents have been reported by Kessler (1981), Dannenberg and Kessler (1988c), de Jong *et al.* (1992), Gotham *et al.* (1992), de Jong and van der Linden (1993), Hinrichs and Kessler (1995), Fryer *et al.* (1995) and de Rafael and Calvo (1996). The operational time of a plate heat exchanger processing fresh liquid milk is longer than when heating recombined milks at the same temperature.

It should be noted, however, that most of the technical data are collated from studies carried out on whole or skimmed milk and, although the various physico-chemical changes will occur in the yoghurt milk, the extent may be dependent on the composition of the milk base.

2.9.4 Processing effects on the physical properties of the gel

It is evident from Section 2.4 that the method of fortification of the milk solids can affect the firmness and syneresis of the yoghurt gel (see also Rohm, 1989, 1993a, b; Rohm and Schmid, 1993; Horne, 1993). Similarly, these same properties are influenced by the homogenisation pressure used (see Section 2.8). However, while the physicochemical changes in the protein components of milk could be considered to be one of the major changes influencing the quality of the manufactured yoghurt, the role of the starter culture in relation to acid development should not be overlooked.

Scanning electron microscopy (SEM) studies on the structure of gels derived from heated and unheated milks revealed some distinctive characteristics of the casein micelles. In heated milks, the gel is formed as the casein micelles gradually increase in size and form a chain matrix. This behaviour results in an even distribution of the protein throughout the yoghurt and the aqueous phase is immobilised within the network; the resultant coagulum is firm and less susceptible to syneresis. On the other hand, the casein micelles in the unheated milk form aggregates or clusters in which the protein is unevenly distributed and this heterogeneity impairs the immobilisation of the water; the coagulum is much weaker, by 50% compared with the previous coagulum (Kalab and Harwalkar, 1973, 1974; Kalab *et al.*, 1976, 1995; Kalab, 1979a, b, 1992; Harwalkar and Kalab, 1980; Modler and Kalab, 1983; Modler *et al.*, 1983). The contrast is well illustrated in Fig. 2.14. Also the casein particles (i.e. chains and clusters) in yoghurt containing 10 g TS 100 g⁻¹ were the largest observed and the dimensions of the particles decreased as the total solids content increased (Harwalkar and Kalab, 1986). In general, the larger the pores in the protein matrix, the easier the separation of whey, whilst the higher resistance to syneresis at pH 3.85 reflected increased gel rigidity compared with yoghurt which had a pH of 4.5.

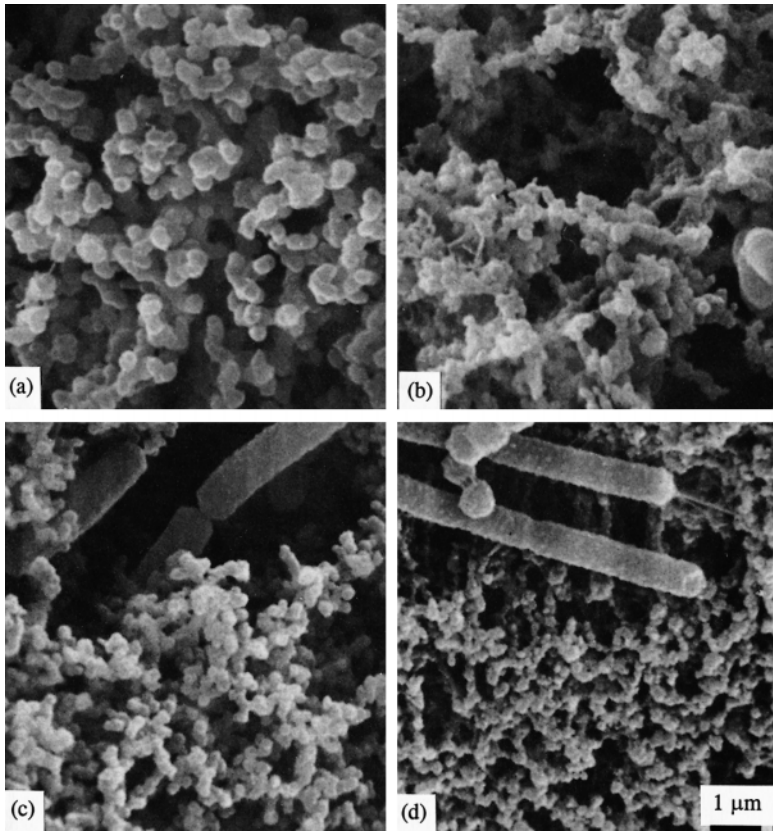


Fig. 2.14 Structural characteristics of the casein in different milks (10 and 20 g 100 g⁻¹ total solids) reconstituted from low skimmed milk powder and heated to 90°C, or unheated for 10 min

(a) Unheated milk (10 g TS 100 g⁻¹), (b) heated milk (10 g TS 100 g⁻¹), (c) unheated milk (20 g TS 100 g⁻¹), (d) Heated milk (20 g TS 100 g⁻¹).

Notice the differences in the structure of the coagulum in unheated and heated milks, (a) and (c) or (b) and (d), and the size of the casein micelle as the level of solids increases in the milk, (a) and (c) or (b) and (d).

After Kalab (1979a).

An investigation of milks subjected to heat (95°C for 10 min) revealed filamentous appendages composed of β -Lg/ κ -casein and the interaction appears to involve —SS linkages and, possibly, the involvement of various salts, for example calcium phosphate and citrates (Davies *et al.*, 1978; Harwalkar and Kalab, 1981, 1988). These appendages tend to become “diffuse” after fermentation, but their presence in the coagulum of heated milks inhibited micellar coalescence, so giving rise to firmer curds with reduced tendencies to syneresis.

The microstructure of yoghurt consists of a protein matrix of micellar short or medium chains and micellar clusters, with the fat globules embedded in the matrix. Both the ratio of casein to non-casein protein in the milk and the method of fortification of the SNF can influence the porosity of the protein matrix. Ratios of 2.9:1 to 4.6:1 have been reported by Modler and Kalab (1983), Modler *et al.* (1983) and Tamime *et al.* (1984). The latter authors recommended a ratio of 3.3:1 because,

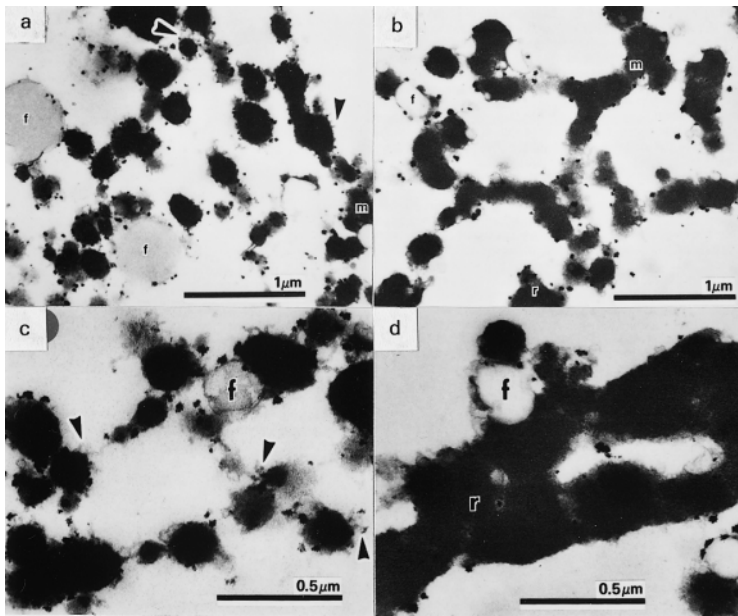


Fig. 2.15 Microstructure (TEM) of casein micelles chains and clusters in yoghurt prepared from skimmed milk fortified with SMP (a) and (c) and Na-caseinate (b) and (d). Arrows point to spikes on casein micelle surfaces. f, Fat globules; m and r, simple and complex casein micelle chains, respectively.

After Tamime *et al.* (1984). Reproduced by permission of *Scanning Microscopy International*.

at higher ratios, fusion of the casein micelles occurred and this resulted in an unsatisfactory product because of textural problems (i.e. being rough and coarse). Figure 2.15 illustrates the microstructure of yoghurt (SEM and TEM) fortified with SMP and Na-caseinate. Incidentally, the yoghurts fortified by VE and RO had similar images to those of SMP, whilst UF yoghurt showed only limited fusion of casein micelles (Tamime *et al.*, 1984). However, using UF retentate powders (whole or skimmed – see Table 2.4) the original ratio of casein to non-casein protein is maintained in the milk base and hence a yoghurt with a firm body and minimal syneresis can be made.

Homogenisation and high-heat treatment of the milk base increases the hydrophilic properties of the coagulum and the stability of the yoghurt gel due to the denaturation of whey proteins and association with κ -casein. Labropoulos *et al.* (1981a, b, 1982, 1984) concluded that the physical properties of yoghurt manufactured from milk heated to 82°C for 30 min, compared with 149°C for 3.3 s, were best, and that the latter treatment is suitable only for the production of drinking yoghurt or yoghurt with thin consistency or low curd firmness. Similar observations were reported by Parnell-Clunies and Kakuda (1986), Parnell-Clunies *et al.* (1986a, b, 1988a) and Parnell-Clunies (1987) for whole milk heated at 85°C for 10–40 min (vat process), at 98°C for 30–112 s (high pasteurisation) and at 140°C for 2–8 s (UHT) for the manufacture of yoghurt. They concluded that:

- The order of yoghurt firmness and viscosity of the three heating methods was vat process > high pasteurisation > UHT treatments, whilst the highest water-holding capacity of the coagulum was observed with high pasteurisation treatment followed by UHT and vat process.
- Yoghurt made by the vat process exhibited syneresis and a grainy texture; UHT treatment resulted in weak texture of the yoghurt coagulum; the high pasteurisation process (i.e. 98°C for 1.87 min) represented the best process and was recommended for industrial production. However, other researchers have recommended 85°C for 30 min for maximum starter activity.

Nevertheless, Schmidt *et al.* (1985) improved the firmness, syneresis and texture of yoghurt by adopting the following method of milk processing. On the first day, separate whole milk at 37°C, fortify with SMP, warm to about 50°C, homogenise at 17.2 MPa (first stage) and 6.9 MPa (second stage) and cool to 4°C for 14 hours. On the second day, heat the milk at 138°C for a longer time than reported by Labropoulos *et al.* (1981a, b), cool to 42°C and inoculate with starter culture and after incubation, cool in ice bath at pH 4.3 and store for 1 week at 4°C. This method, if adopted for the manufacture of yoghurt, may be inconvenient for industrial application and the consensus in the reported literature suggests that high pasteurisation of the yoghurt milk is most suitable (Dannenberg and Kessler, 1988d, e; Mottar *et al.*, 1989; Kessler *et al.*, 1990; Kaytanli, 1993). It may also be important that, upon heating the milk base, the microstructure of the heated micelles showed large numbers of small particles of irregular shape attached to the micelle surface (Fig. 2.16a) and finely

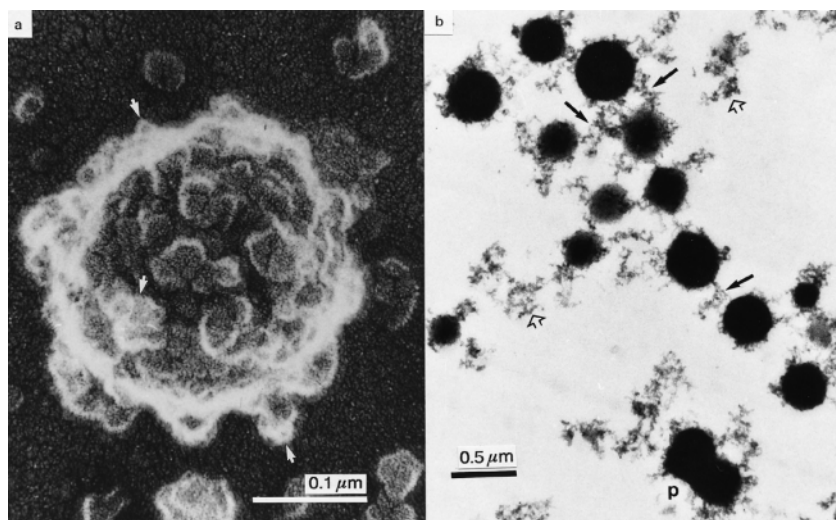


Fig. 2.16 Casein micelle (a) in heated bovine skimmed milk covered with corpuscular spikes (arrows) using rotary shadowing, and casein micelles (b) in milk fortified by UF-WPC appear as individual entities in a loose chain attached to finely flocculated protein (dark arrows) and some not associated with the micelles (light arrows). Occasionally some micelles (P) are tightly fused together

After Kalab *et al.* (1982) and Modler and Kalab (1983). Reproduced by permission of *Milchwissenschaft* and *Journal of Dairy Science*.

flocculated protein surrounding the casein micelles (or as separate entities) (Fig. 2.16b) in yoghurt milk fortified with UF-WPC (Kalab *et al.*, 1982, 1983; Modler and Kalab, 1983; Kalab and Caric, 1990; Kalab, 1992). Recently, Hollar *et al.* (1995) dialysed a WPC mixture (16gTS/100g⁻¹) against simulated UF milk containing calcium; on heating, the denaturation of the whey proteins was influenced by:

- the calcium content which, as it decreased, resulted in more soluble aggregates and less soluble precipitates being formed;
- the pH which, as it increased (5.8 to 7.0), caused more protein denaturation, fewer soluble aggregates and more soluble precipitates;
- α -La was denatured more extensively than β -Lg at 66°C and 71°C;
- the addition of low-heat SMP limited whey protein denaturation in WPC.

Another aspect, which was revealed by SEM studies (Kalab, 1979a), showed that *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* form “pockets” in the protein matrix of the yoghurt coagulum. These pockets were regarded by some workers in this field as artefacts caused by freeze drying of the sample, but both transmission electron microscopy (TEM) and the freeze fracturing of yoghurts, that is, sectioning while the aqueous phase is still present, confirmed the presence of the pockets (Kalab, personal communication). Figure 2.14 shows some lactic acid bacteria in a “void space”. Furthermore, SEM micrographs (Kalab, 1979b, 1992, 1993) also revealed filaments of exopolysaccharide (EPS) produced by “slime” or “ropy” strains of yoghurt starter cultures (see also Brooker, 1979, 1987). Further detail regarding the chemical composition of these filaments is given in Chapter 7.

The dimensions of the casein particles in yoghurt milk are affected by the level of total solids in the milk base, and Kalab (1979b) observed that the size of the casein particles decreased with increasing levels of solids in the milk (see Fig. 2.14); the reason for this behaviour is not well established.

Image analysis using TEM was used by Skriver *et al.* (1997) to study the microstructure of yoghurt and they observed that (a) casein aggregates were larger in the yoghurt made from milk heated at high temperature, so confirming the observations reported by Kalab *et al.* (1976), (b) the star volume, which gave a measure of the average size of “pores” in the yoghurt gel, was influenced by the level of heat supplied to milk and also affected the structure of the casein matrix and (c) the covariance function was able to differentiate between yoghurts made from the same heated milks, but held for 10 min or 30 min.

Normal creaming in cold milk is influenced by the action of the globulins, which assist in the formation of clusters among the rising fat globules (Mulder and Walstra, 1974). Therefore, the denaturation of the globulin fractions in milk, as a result of heat treatment, causes a reduction in the cream layer (Walstra and Jenness, 1984). This action could work in favour of the small yoghurt producers whose production lines do not include an homogeniser. Furthermore, milk becomes whiter in colour on heat treatment, before the appearance of browning and according to Burton (1954) this could be due to:

- flocculation of the whey proteins;
- changes in the casein aggregates;
- calcium being converted from the soluble state to a colloidal or insoluble form.

2.10 Fermentation process

2.10.1 Introduction

During the manufacture of yoghurt, the heat-treated milk is cooled to the incubation temperature of the starter culture (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) and, in general, the milk is fermented at 40–45°C, that is, the optimum growth condition for the mixed culture – the short incubation method. In some cases the incubation period can be as short as 2½ hours, assuming that the starter culture (3%) is an active one and the ratio between the rods and the cocci is well balanced. However, the longer incubation method, (i.e. overnight) can be used and the incubation conditions are 30°C for around 16–18 hours, or until the desired acidity is reached (Hrabova and Hylmar, 1987). Recently, zabadi (i.e. Egyptian equivalent of yoghurt) has been manufactured at 30°C or 35°C with minimum syneresis, improved firmness and smoothness and with a pleasant flavour (Mehanna, 1991). However, Cho-Ah-Ying *et al.* (1990) produced yoghurt using different strains of thermophilic starter cultures (i.e. EPS (exopolysaccharide) producer and non EPS-producer), and fermenting the milk at 38° and 43°C; they concluded that the temperature of incubation had significantly affected only one sensory character (e.g. texture), and overall the yoghurts made at 38°C had the tendency to score higher.

While the cooled milk is being pumped to the fermentation tanks, the starter culture is normally metered directly into the milk, or alternatively, if a multipurpose tank is being used, the starter culture is added either manually or, if the volume of the tank is large, the desired quantity of starter is pumped into the tank. As can be seen later, the actual fermentation stage can take place either in the retail container for the production of set yoghurt, or the milk is incubated in bulk for the manufacture of stirred yoghurt. However, no matter what type of yoghurt is being produced, the biochemical reactions responsible for the formation of the gel/coagulum are exactly the same. The intricacies of the fermentation processes are discussed in detail in Chapter 7. Thus, the only real differences between set and stirred yoghurt are the rheological properties of the coagulum, since in the former type the milk is left undisturbed during the incubation period and the resultant gel is in the form of a continuous semi-solid mass, while stirred yoghurts are, by contrast, the result of breaking the gel structure at the end of the incubation period and prior to cooling and further processing (refer to Chapter 5 for more detail).

2.10.2 Starter organisms

The commercial process of yoghurt making uses a defined mixture of lactic acid bacteria, for example *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, but other products may require a different blend. For example, Bulgarian buttermilk is produced using *L. delbrueckii* subsp. *bulgaricus* alone, whilst dahi in India is produced using a mixed starter culture containing *S. thermophilus*, *Lactococcus lactis* biovar *diacetylactis* and *Lactococcus lactis* subsp. *cremoris* (Tamime and Marshall, 1997). Bio-yoghurts are made with different and defined starter cultures containing the yoghurt organisms (single or mixed) and/or *Lactobacillus*, *Bifidobacterium* and *Enterococcus* species; for further detail refer to Chapter 5 and Tamime and Marshall (1997).

The reason(s) for selecting the combinations of starter cultures used during the manufacture of yoghurt and related fermented milk products are to achieve the

desired flavour characteristics of the product, mainly lactate, aroma compounds (acetaldehyde, acetoin and diacetyl) and EPS and to provide the consumer with a wide choice of therapeutic products. The former aspect is very important and, hence, careful selection of different strains of the yoghurt organisms may provide the manufacturer with the following broad options of flavour intensity and EPS production by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*:

Flavour	EPS
High	Low
Medium	Medium
Low	High

Although the low flavour producing strains tend to be categorised as high EPS producers, the same pattern may emerge organoleptically because the presence of EPS in a yoghurt may mask the flavour attribute of the product; such products will also have a different mouthfeel (Wacher-Rodarte *et al.*, 1993). Rohm (1992, 1993a, b), Rohm and Kovac (1994, 1995), Rohm *et al.* (1994) and van Marle and Zoon (1995) studied the textural and organoleptic characteristics imparted by a number of commercially available yoghurt starter cultures strains and they concluded that:

- Significant differences between the yoghurts were found for each sensory attribute except gel firmness.
- Multiple regression analysis of the sensory scores obtained using the hedonic scale were mainly influenced by the ropiness and flavour attributes showing negative and positive weightings, respectively.
- The apparent viscosity of stirred yoghurt was increased by the EPS cultures, but no correlation was observed between viscosity and the amount of EPS produced; the permeabilities of glucon- δ -lactone (GDL), EPS and non-EPS gels, as measured by using glass tubes (van Marle and Zoon, 1995), were significantly different, and the lowest value was shown by the EPS gel; thus, the permeability and apparent viscosity of stirred gels are inversely related.
- The relationship between shear stress and long relaxation time was more evident in the viscoelastic properties of products made with EPS cultures.

This latter aspect could be influenced by the attachment of ropy bacterial cells to the protein matrix, which thus decreased the firmness of the yoghurt gel. Figure 2.17 illustrates some examples of the microstructure of yoghurt made with EPS-producing starter cultures. Incidentally, a similar microstructure has been reported by Toba *et al.* (1990) for viili, a Finnish fermented milk, made with a ropy strain of *Lactococcus* species.

Schellaass (1983) and Schellaass and Morris (1985) observed that yoghurt made with EPS cultures exhibited a decreased susceptibility to syneresis and greater viscosity when compared with non-EPS producer strains (Robinson, 1988); however, excessive EPS production was obtained when the milk was fermented at 32°C. Such physical characteristics of the EPS yoghurt are attributed to the filamentous network between the bacterial cells and the casein matrix, but this interaction was disrupted when the yoghurt was subjected to shear stress at 220 s⁻¹ (Teggatz and Morris, 1990; Skriver, 1995; Skriver *et al.*, 1995). Hassan *et al.* (1995a, b) have used confocal scanning laser microscopy (CSLM) to observe the structure of yoghurt in its natural state and they observed that: (a) an envelope of EPS produced by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was evident surrounding the cells, (b) the diameter of

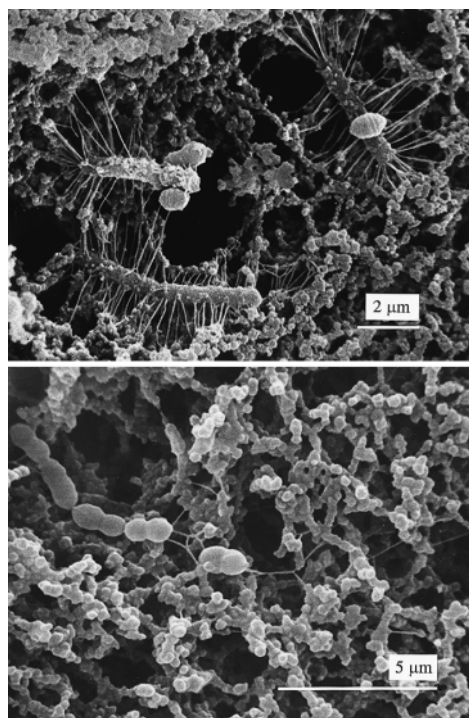


Fig. 2.17 Microstructure of yoghurt made with an exopolysaccharide (EPS) strain of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*

After M. Kalab (personal communication).

the envelope was different depending on the bacterial species and (c) gelation of the milk, as determined by casein micelle aggregation and the cessation of bacterial cell movement, was initiated at pH 5.35; as the pH continued to drop, the EPS organisms caused the formation of non-reflective zones in the gel possibly due to the contraction of the casein matrix. The textural and rheological properties of yoghurt made with these cultures have been reported by Hassan *et al.* (1996a, b).

It is relevant to point out that very simple techniques can also be employed to study the behaviour of EPS yoghurt, such as monitoring the syneresis of yoghurt on an inclined heated black glass (Tamime, 1977) and measuring the length of an EPS “thread” formed at the end of an acrylic rod when withdrawn from yoghurt (Watanabe, 1987).

Advance(s) in yoghurt technology may include the interactive fermentation of milk by means of a membrane dialysis fermenter. Such a fermenter has been developed in The Netherlands (Klaver *et al.*, 1992a) to obtain a yoghurt with a smooth structure, mild acidity and lower post-fermentation acidification during storage. The same researchers have also used the technology in buttermilk making (Klaver *et al.*, 1992b). An alternative approach to limiting acid development and bitter taste in yoghurt during storage has been reported by Klaver *et al.* (1991), and the system could be described as follows: (i) the processed milk is cooled to the incubation temperature and fermented by *Lactobacillus* sp., followed by a heat treatment to inactivate the starter cultures, and (ii) the partially fermented milk is cooled and inoculated with streptococci; sweet or unfermented milk could be added as optional

extra. In Egypt, El-Kenany *et al.* (1996) inoculated milk with a yoghurt starter culture at 60°C or 70°C with a holding time of 5 min, before cooling to 45°C; this method of fermentation improved the shelf life of the product.

2.10.3 Gel formation

The formation of gels during the manufacture of certain dairy products is basically due to destabilisation of the casein complex. These gels are irreversible and are classified into different groups: (a) enzymic gels, which are formed as the result of coagulant action which destabilises the κ -casein allowing aggregation of the casein in the presence of calcium ions, (b) heat-induced gels, which can arise as a fault where gelation occurs in UHT milk or evaporated milk if the protein fraction is not well stabilised, (c) acid gels formed by the acid fermentation of milk, for example yoghurt, and (d) salt/heat-induced gels, which are normally produced during the manufacture of Ricotta cheese. It could be argued, however, that, although the production of yoghurt does not involve the addition of a proteolytic coagulant enzyme, proteinases originating from the yoghurt starter cultures may have a role. Hence, it should be understood that yoghurt may not be simply an acid-induced gel and that proteinases may contribute to the denatured protein matrix which could be relevant to the gel properties of yoghurt (Tamime and Marshall, 1997).

The main difference(s) between acid- and enzymic-induced milk gels have been reported by Walstra and van Vliet (1986) and van Vliet *et al.* (1989) and could be summarised as follows: (a) The permeability of the acid-induced gel does not change during the first 24 hour after gelation, whilst in an enzymic-induced gel, it increases continuously during the same period, and (b) a milk gel formed by coagulant enzymes is more robust than an acid-induced gel; the latter type of gel is fragile and shatters very easily. Nevertheless, Muir and Hunter (1992) have profiled the sensory attributes (i.e. *odour*: intensity, sour, fruity, buttery, yeasty, creamy, sweet, other; *flavour*: intensity, sour/acid, fruity, buttery, rancid, creamy, salty, bitter, lemon, sweet, chemical, other; *aftertaste*: intensity, bitter, sour/acid, other; *texture*: firmness, creaminess, viscosity, sliminess, curdy character, mouthcoating, chalky, serum separation) of fermented milks, and also identified these attributes as being important for consumer acceptability. The microstructure of yoghurt has been well studied, but few data have been published on the mechanism(s) of the acid induction of gels in milk by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* at 30–45°C. However, the casein micelles are composed of different protein fractions (see Section 2.9.3), and are associated with one another via Ca-phosphate bridges. During the fermentation of milk, the micellar or colloidal Ca^{2+} content (and possibly to a lesser extent magnesium and citrate) increases in the serum as the pH is lowered due to the solubilisation of micellar Ca-phosphate (Pouliot *et al.*, 1989; LeGraet and Brulé, 1993). Alteration of the physical nature of the casein micelles will play a major role in acid-induced milk gels.

The mechanism(s) of dissociation and aggregation of casein micelles in acid-induced gels has been reviewed by Tamime and Marshall (1997) and they reported that:

“direct acidification of milk using HCl or glucono- δ -lactone (GDL) and the addition of calcium chelating agents are different techniques used to study the gelation of milk under controlled conditions without the metabolic interference of the starter

cultures (Roefs *et al.*, 1985; Holt *et al.*, 1986; van Hooydonk *et al.*, 1986; Visser *et al.*, 1986; Roefs, 1987; Bremer *et al.*, 1990; Bringe and Kinsella, 1990, 1991; Banon and Hardy, 1991, 1992; Horne and Davidson, 1993).

Studies of casein micelle dissociation and aggregation during the acid-induced gelation of milk suggest that the mechanisms involved are pH, -ion concentration- and temperature-dependent (Aoki *et al.*, 1986, 1987a, b, 1988; Holt *et al.*, 1986; Singh *et al.*, 1996b; Teo *et al.*, 1996, 1997). Predominantly, β -casein dissociates from the casein micelles at low pH (Hooydonk *et al.*, 1986); however, dissociation of other casein fractions (κ -, α_{s1} -, α_{s2}) from the micelles has been reported by Roefs *et al.* (1985), Roefs (1987), Dalgleish and Law (1988) and Ward *et al.* (1996). All these authors also observed that the amounts and proportions of the dissociated caseins in the serum were pH- and temperature-dependent. At pH 5.6, all the major caseins were prone to dissociation and the dissociation occurred at the outer rather than the inner layers of the submicelles (van Hooydonk *et al.*, 1986). Solubilisation of the micellar Ca-phosphate occurs at pH \leq 5.3, and there is a linear relationship between $\text{Ca}^{2+} + \text{Mg}^{2+}$ and inorganic phosphate (P_i) + citrate (cit.). The binding of ionic calcium and magnesium to casein appears to be independent of pH between 5.6 to 6.7. Calcium binding may involve carboxyl groups; however, a decrease in pH also affects the spatial properties because of electrostatic interactions between positively and negatively charged groups (van Hooydonk *et al.*, 1986). Dalgleish and Law (1989) observed a similar pattern of mineral solubilisation due to pH- and temperature-induced conditions, but could not suggest a universal relationship to describe the dissociation of salt ions and the caseins from the micelles. Lowering the pH reduces the repulsive forces and allows for hydrophobic interactions causing the casein micelles to coagulate. However, preheating the skimmed milk to 90°C followed by acidification at 30°C using slow hydrolysis of GDL shifted the coagulation pH to a value higher than 5.5, and shortened the coagulation time (Horne and Davidson, 1993; Cobos *et al.*, 1995a, b).

Quiescent heating of casein solutions made with reconstituted SMP or Na-caseinate, and acidified at 0–2°C produced physically stable suspensions of casein particles (Roefs *et al.*, 1990a, b). Gelation occurred above 10°C and lowering the temperature to 4°C after gel formation had the following effects: (a) the casein particles formed a complex irreversible structure, (b) the acid-induced gel was formed subject to an activation Gibbs energy which decreased on increasing the temperature, (c) if the gel was good at $>10^\circ\text{C}$, the dynamic moduli, G' and G'' , linearly increased with the logarithm of time over at least a week, and (d) the gel network consisted of large and small agglomerates of casein particle aggregates in the form of strands and nodes, with void spaces around 1–10 μm ; this suggests that the strands and nodes are made of concentrated protein (about 25%) with a modulus of about 10^5 N^{-1} (Roefs *et al.*, 1990a, b). Similar gel characterisation of commercial fractions of β -Lg and α -La have been recently reported by Rojas *et al.* (1997).

The dissociation of casein micelles in milk has been induced by other means such as salt solutions (CaCl_2 , MgCl_2 or NaCl) or calcium-chelating agents like EDTA, hexametaphosphate, oxalate, citrate or othophosphate (Holt *et al.*, 1986; Aoki *et al.*, 1988; Rollema and Brinkhuis, 1989; Bringe and Kinsella, 1991; Johnston and Murphy, 1992; Goddard and Augustin, 1995; Goddard, 1996). Dialysis against phosphate-free and Ca-phosphate buffers decreased both the colloidal Ca phosphate and P_i (depending on the type of buffer used) before casein dissociation occurred. Holt *et al.* (1986) reported that dissociation resulted from the breakup of

linkages between the casein and the inorganic components. However, the dissociation of casein micelles in simulated milk ultrafiltrate dialysed against imidazole buffer was dependent on the ester phosphate content (Aoki *et al.*, 1988). The addition of Ca^{2+} , Mg^{2+} and Na^+ ions, which are associated with casein phosphates and carboxyl groups, tends to increase the hydrogen ion concentration due to reduced repulsive hydration forces between micelles. Hence, the attractive hydration forces cause coagulation because hydrogen ions displace bound Ca^{2+} , Mg^{2+} and Na^+ ions in the casein micelles. Ions (Cl , NO_3 , Br and SCN) binding to lysine, arginine and histidine groups also decrease the repulsive hydration forces between colloidal ions of the casein micelles (Bringe and Kinsella, 1991).

Current published research on the mechanism(s) of gelation induced by direct acid addition to milk provides some limited information; however, since the milk base for the manufacture of fermented milks is prepared in a different way and subjected to homogenisation and high heat treatment, the properties of the fermentation-induced gel may differ. Roefs *et al.* (1985) concluded that, because low-heat SMP was used in their study: 'because of the dependency of results on the history of samples, both in terms of pH and temperature, it will take painstaking studies to determine precisely what changes occur'.

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It is evident that the formation of yoghurt gel is the result of both biological and physical action on the milk, such as the fortification, homogenisation and heat treatment of the milk base and the catabolism of lactose in the milk by the starter culture for its energy requirements and, as a result, the production of lactic acid and other compounds. These effects bring about the gelation of milk. Heertje *et al.* (1985) reported that, during the acidification of skimmed milk with GDL at 30°C, the casein micelles may undergo the following changes at different pHs (see also Mulvihill and Grufferty, 1995):

- 6.6–5.9, no evidence of change in the casein micelles, size about 0.1 μm and homogeneously distributed in milk.
- 5.5–5.2, partial micellar disintegration occurs and at ≤ 5.2 , casein particles aggregate to form structures with empty spaces between them; however, when such interaction(s) between micelles take place, the milk gel should not be disturbed.
- 5.2–4.8, contraction of casein aggregates take place, and these particles are larger in size than the native micelles.
- ≤ 4.5 , rearrangement and aggregation of casein particles occurs leading to the formation of a protein matrix consisting of micellar chains and clusters.

Parnell-Clunies *et al.* (1988b) concluded that acid-gel formation of milk was a multi-stage process consisting of an initial lag period of low viscosity, a period of rapid viscosity change and a stage of high viscosity. However, the same authors reported that dissociation of casein micelles occurred at pH 5.1 and was thought to be influenced by the conversion of colloidal Ca to Ca^{2+} . At pH 4.8 these casein sub-particles reassociate to form larger casein aggregates bearing no specific shape and dimensions.

Overall it is reasonable to conclude and/or suggest that the α -La/ β -Lg interaction with the κ -casein (linked by —SH and —SS bridges) partially protects the micelles; however, as the pH in milks is lowered, destabilisation or disruption of the

micelles starts to occur. As a result, the gel network or protein matrix consists of micellar chains and/or micellar clusters and entraps within it all the other constituents of the milk base, including the water phase.

2.11 Cooling

Yoghurt production is a biological process and cooling is one of the popular methods used to control the metabolic activity of the starter culture and its enzymes. Cooling of the coagulum commences directly after the product reaches the desired acidity, for example, around pH 4.6 or 0.9% lactic acid depending on the type of yoghurt produced, the method of cooling used and/or the efficiency of heat transfer.

Since the yoghurt organisms show limited growth activity around 10°C, the primary objective of cooling is to drop the temperature of the coagulum from 30–45°C to <10°C (best at around 5°C) as quickly as possible so as to control the final acidity of the product. The process of cooling yoghurt may be carried out using one-phase or two-phase cooling.

2.11.1 One-phase cooling

In this process the coagulum is cooled directly from the incubation temperature to <10°C prior to the addition of flavouring materials and packaging. This approach is based on the assumption that a cold coagulum is more stable than one at about 20°C, and hence less damage will occur during the subsequent stages (e.g. mechanical handling while introducing the fruit/flavours, and filling the retail cartons). In actual fact, the coagulum at about 20°C is less viscous and, as a consequence, the product can be transferred from one section of the processing equipment to another with minimal structural damage. Thus, one-phase cooling is not widely used in the industrial situation.

2.11.2 Two-phase cooling

The first phase of the cooling stage reduces the temperature of the coagulum from 30–45°C to about 20°C prior to addition of the flavouring materials and filling. The second phase of cooling takes place in the refrigerated cold store where the yoghurt is cooled to <10°C. The final cooling of yoghurt takes place, therefore, in the retail container and as the coagulum is left undisturbed, the viscosity of the yoghurt improves after 1–2 days' storage.

This latter approach to cooling is widely used in the industry for the production of acceptable viscous yoghurts. However, the influence of cooling rate on the physical characteristics of stirred yoghurt was evaluated at the Danish Dairy Research Institute (Anon., 1977) and they gave the following recommendations:

- The quality of stirred yoghurt may be greatly improved by packaging yoghurt at 24°C, followed by final cooling of the product in the container.
- To achieve the maximum effect on yoghurt quality, the second-phase of cooling must be carried out as slowly as possible over a 12 hour period.

- Concentration of the yoghurt milk, i.e. by evaporation and removal of about 10% water, was identified as the factor that most improved the quality of yoghurt.
- The recommended procedure was as follows: (a) before cooling commences, stir the yoghurt in the incubation tank until mixture is homogeneous, (b) cool the yoghurt (primary cooling) to 24°C and package, (c) cool the packed yoghurt in a cold store controlled by a two-step temperature regulator, i.e. the first 5–6 hours at an air temperature of 7–10°C, and then at an air temperature of 1–2°C for the remainder of the cooling period, (d) forced air circulation in the cold store is highly recommended to obtain uniform cooling of the packaged yoghurt and (e) the design and construction of the crate and the material(s) used for packaging can affect the cooling rate of the packed yoghurt.

However, the industry does not appear to have adopted these proposals to any marked extent. The general practice in large installations is to subject the packaged yoghurt to an intermediate shock cooling in a chill tunnel before reaching the refrigerated cold store (Bylund, 1995). White (1995) has described a multistage cooling process for yoghurt which entails the following basic phases:

- Shock cooling from 42°C to 30°C.
- Dysgential stage from 30°C to 20°C.
- Lact-less phase to 14.5°C.
- Holding phase at 2–4°C.

This approach could be considered as a slight modification of the system described by Bylund (1995), but it may be difficult to adapt to industrial situations unless some of the stages are combined before the product is packaged.

In addition, it should be noted that the cooling of yoghurt starts at a relatively high pH value, and hence the rate of cooling (slow or fast) determines the final acidity in the product and the rate of cooling can affect the structure of the milk gel. Very rapid cooling may lead to whey separation, possibly due to a too rapid contraction of the protein matrix which, in turn, affects the hydrophilic properties (Rasic and Kurmann, 1978).

2.12 Addition of fruit/flavouring/colouring ingredients

The increase in the per capita annual consumption of yoghurt in the majority of countries (see Table 1.2) has been attributed both to the ever-increasing availability of fruit and/or flavoured yoghurts and to the diversity of presentation of the product. Thus, in the United Kingdom, for example, the retail economic value of yoghurt increased from £103m in 1981 to £401m in 1990, reflecting a growth of 3.9-fold (Anon., 1984a, 1991a); 90% of these sales are fruit and flavoured yoghurts.

A variety of different flavouring ingredients (fruits, natural flavours and/or synthetic flavours) are currently added to yoghurt and Table 2.21 indicates a range of available fruit additives. It can be observed that the fruit flavours, which are in regular demand, are surprisingly few in number, and the rest are introduced by the yoghurt manufacturers merely to encourage wider popularity for the product. The types of fruit/flavouring material used in the yoghurt industry are given below.

Table 2.21 Fruits and fruit flavours currently used in production of yoghurt

Regular demand	Average demand	Poor demand
I. Single		
Apricot	Banana	Apple
Black cherry	Bilberry	Bramble (artic)
Blackcurrant	Blackberry	Cranberry
Mandarin	Gooseberry	Damson
Peach	Grapefruit	Elderberry
Pineapple	Lemon	Grape
Raspberry	Melon	Guanabana
Strawberry	Orange	Guava
	Plum	Kiwi
	Prune	Kokum
	Rhubarb	Lime
	Tangerine	Loganberry
	Toffee	Mango
		Papaya
		Passion fruit
		Pear
		Pina Colada
		Quince
		Redcurrant
		Sapota
		Wortleberry
II. Mixed		
Fruit cocktail	Apple/Raisin	Apple/Wortleberry
Fruit of the Forest	Apple/Orange	Cherry/Elderberry
Peach/Raspberry	Cherry/Orange	Grape/Figs
Peach/Apricot	Cherry/Pineapple	Kiwi/Gooseberry
Raspberry/Redcurrant	Mixed Citrus	Peach/Passion fruit
	Pear/Banana	Pineapple/Coconut
	Strawberry/Blackberry	Raspberry/Coconut
		Strawberry/Kiwi
		Strawberry/Coconut
III. Miscellaneous flavours		
Almond paste/Low-starch nuts		Mint
Banoffee		Mirabella/Vanilla
Buckwheat Honey		Mississippi Mud Pie
Butterscotch		Mocca
Champagne		Muesli
Chocolate		Muesli/(ω)-3 fatty acids
Chocolate covered crisps		Nuts/Cocoa, Caramel or Nougat
Coconut		Paprika/Celery
Coffee		Pear/Vanilla
Cucumber		Raspberry/Vanilla
Grape Mist		Tomato
Hazel nuts		Vanilla
Honey		Walnut
Lemon Meringue Pie		Wine
Maple syrup		

The classification of the above fruit/flavoured yoghurt is only applicable to the U.K. market as suggested by Tamime and Hamilton (unpublished data).

Data compiled from Brown and Kosikowski (1970), Swanson *et al.* (1972), Robinson and Tamime (1975), Duthie *et al.* (1977), Muller (1977), Bray *et al.* (1979), Chase (1981), Lang (1981a, b), Anon. (1981a, 1984b, 1986a, b, 1987a), Lehmann (1983), Kammerlehner (1986), Matsunobu *et al.* (1987), Schei and Abrahamson (1987), Herner (1987a, b), Payne (1988), Kaufman *et al.* (1990), Balasubramanyam and Kulkarni (1991), Jarmarova and Hrabe (1992), Hoang (1992), Raiski (1993), Desai *et al.* (1994), Calvo *et al.* (1995) and Mortensen (1996).

2.12.1 Fruits

Fresh fruits can be used to flavour yoghurt, but due to the seasonal availability of such materials and their variable quality, their use in the industry is very limited. Processed fruits are, therefore, more widely employed, particularly as the desired fruit mixture can be standardised by the fruit processor to meet the specifications required by the customer. In general, fruit preparations for the yoghurt industry consist of fruit, sugar (syrup and/or artificial sweeteners), stabilisers, flavours, colouring matter and food grade acids or pH adjusters (see Hegenbart, 1990; Mogensen, 1995). These types of fruits are classified as fruit preserves, canned fruit, frozen fruits or miscellaneous fruit products.

2.12.1.1 *Fruit preserves*

Fruit preserves are processed in a small quantity of sugar syrup to give an end product consisting of ($\text{g } 100 \text{ g}^{-1}$) 70 fruit, 30 water, and this product may be referred to as pure or natural, since no colouring matter or preservatives are added. Depending on the processing technique, the product may become highly aromatic, but the natural colours of any fruit become dull due to the effect of heat treatment. It is also relevant that such products are expensive, so that overall demand from the yoghurt industry is limited.

2.12.1.2 *Canned fruit*

Canned fruit is similar to the product mentioned above, except that canned fruits are permitted to contain certain additives, such as (a) colouring ingredients which help to mask the loss of the natural colours of the fruit, (b) stabilisers which assist in protecting the structure of the processed fruit and improve the viscosity of the fruit product, and (c) flavouring agents which help to enhance the consumer appeal of the finished yoghurt.

Canned fruit is packaged in special lacquered tin cans, plastic drums with polyliners, laminated plastic bags (Mora, 1996) or stainless steel tanks. The level of sugar is maintained at $30\text{--}35 \text{ g } 100 \text{ g}^{-1}$ and the pH is adjusted to <3 , and although this latter factor helps to protect the product against spoilage, it may lead to minor problems of whey separation. Different time/temperature conditions are used for the heat treatment of the various fruits and the microbiological specifications of such products can either be “sterile” or to standards proposed by the fruit processors (see Chapter 10). Although the processing of fruit is sometimes carried out by large dairy organisations, the majority of yoghurt manufacturers rely on specialist fruit processors.

2.12.1.3 *Frozen fruits*

Frozen fruits are stored at around -20°C for use whenever required. The product is then thawed, sweetened and finally heat treated and, depending on the acidity of the fruit, the temperature of the heat treatment can vary from as low as 60°C to as high as 95°C . Since the freezing process can damage the structure of the fruit, care must be exercised to minimise injury, that is, by harvesting the fruit at a certain degree of ripeness, quick freezing and/or the addition of stabilisers during the heating stage. Colouring matter is sometimes added during processing to offset the browning reactions (enzymatic or oxidative) that can occur during thawing/subsequent heating. The final processing of frozen fruit can be carried out at the dairy, an approach which may be attractive in large-scale factories.

A recent development in fruit processing is the use of the 'osmodehydrofrozen' process which consists of osmotic treatment in sugar solution, limited air dehydration to reduce A_w and freezing and storage (Torreggiani *et al.*, 1988). Fruits processed using this technique require no preservatives, maintain their natural flavour and colour and have an acceptable texture (Erba *et al.*, 1994). Furthermore, when such fruit(s) or dried pieces (Mastrocola *et al.*, 1997) are added to yoghurt, they have the tendency to absorb some of the free or unbound water from the yoghurt gel and hence help to reduce whey separation of the product during storage. Giangiacomo *et al.* (1994) reported that the sensory properties of yoghurt with added osmodehydrofrozen apricot or peach cubes of high solids content significantly improved the consistency of the product; enhanced rehydration properties of osmodehydrofrozen fruits have been achieved in the presence of sorbitol in the syrup (Erba *et al.*, 1994).

2.12.1.4 Miscellaneous fruit products

These may include (a) fruit puree which is homogenised to give an end product in the form of a paste; the shape of the fruit is lost altogether and the fibrous material may also be removed, (b) fruit syrup which is a clear product devoid of solid contents but with a sweetening agent added to it and used during the manufacture of flavoured set yoghurt or drinking yoghurt; in set yoghurt, the syrup is added to the inoculated milk before the packaging and the incubation stages, but for drinking yoghurt, the syrup could be added to the cold natural yoghurt, and (c) jam which is used only during the manufacture of certain types of set yoghurt or in the absence of other processed fruit sources. It is not advisable to add jam to stirred yoghurt since the high viscosity of jam may make it difficult to mix properly with the natural yoghurt; prolonged mixing can result in whey separation or a reduction in the viscosity of yoghurt. However, if jam is used to flavour set yoghurt, a special metering device must be installed on the filling machine so that the required amount of jam is deposited in the carton before it is filled with the inoculated milk.

Low sugar jams can be made with a combination of artificial sweeteners; however, xylitol and sorbitol affect the texture and the use of maltodextrin as a bulking agent can affect the appearance and taste of the product (Hyvonen and Torma, 1983a). The keeping quality of such jams has been studied by Hyvonen and Torma (1983b) and they reported that: (a) jams made with sorbitol and xylitol were of good quality and similar to sucrose-based jam, (b) there was a deterioration in colour, taste and preference during storage of fructose and high fructose jams but, in presence of xylitol, these defects were minimised, and (c) crystallisation and deterioration in the quality of the xylitol-maltodextrin jams was observed during storage.

Bulk fruits (i.e. fresh, sulphited or frozen) have already been cleaned from foreign matter (vegetable matter, insects, stones, metal or sticks) and mouldy, blemished or unripe fruit removed before processing. However, pesticide residues, general microbiological standards and the presence of undesirable additives have to be considered before accepting fruit for processing. A survey of 252 samples of fruits to be used in yoghurt for insect fragments was reported by Locatelli (1988) who found that 15% of large fruits and 58% of berries had been contaminated with insects due to post-harvest contamination.

The heat treatment of the fruits is carried out either in a batch process at 85°C for 10 min or a continuous process at 100°C (flash) (G. Spinks, personal communication; Spinks and Davey, 1970; Szemplenski, 1981). It is important that the tank for

batch processing is designed to minimise fruit damage during heating, whilst scraped-surface heat exchangers are used in the continuous method (see also Sommi, 1996). Some future developments in the processing of fruit may include ohmic heating, high pressure or microwave heating (Langley-Danysz, 1996) and irradiation (Kiss, 1975) but their use is limited at present. However, hot filling of the processed fruit into sterile containers can extend the shelf life of the product without the addition of preservatives (Kivi, 1981; Eller, 1988; Ehrhardt, 1991; Anon., 1993c), as can the use of nitrogen for gas flushing/modified atmosphere packaging (Anon., 1993b).

Some recommended processes for fruits may include (a) stabilisation of the product using apple pectin (Weiss, 1983), or a mixture of low-methoxy pectin and xanthan (Leipold, 1983), or hydroxypropylated starches (Walter, 1996) or amidated pectin that has been standardised with Ca^{2+} salt and carob seed meal (optional) (Kratz and Dengler, 1995a–c), (b) treatment of cereal(s) and/or museli with a water-in-oil emulsion before mixing with yoghurt maintains the crispy texture (Kaufman *et al.*, 1990), (c) soaking peach slices in a solution of calcium chloride ($0.3 \text{ g } 100 \text{ g}^{-1}$) before the heat treatment stage retains flesh firmness (Kim and Choi, 1983), and (d) addition of chopped raisins at a rate of $10 \text{ g } 100 \text{ g}^{-1}$ in yoghurt was highly rated by consumers in Chile, but depended on the use of a special variety rather than any type of raisin (Nicholls *et al.*, 1984).

2.12.2 Flavouring agents

The heat treatment of fruit preparations can result in a reduction in their flavour intensity and hence it is the practice to add flavouring agents to compensate for such losses (Nursten, 1982; Werry, 1982; Heath, 1983; Cowle, 1985; Hudson, 1986; Hodrien, 1990; Jaubert, 1992; Fisher and Scott, 1997). Flavouring agents are divided into three categories depending on their source:

- natural flavours and flavouring substances (botanical origin),
- nature-identical flavouring substances (botanical origin),
- artificial/synthetic substances (chemical origin).

Although the above classification may seem simple, in actual fact the list of possible agents can run into thousands. Flavouring compounds of chemical/synthetic origin are sometimes used due to their provision of a flavour similar to that of a natural ingredient (see Table 2.22), but the list of permitted compounds varies from one country to another. In the United Kingdom, the SI (1995) contains a list of flavouring agents which could be added to food; however, according to the FAO/WHO (1990), no list needs to be provided regarding the artificial flavouring substances that are permitted for use in fruit and flavoured yoghurts and related products. Instead, the maximum level of use is limited by good manufacturing practice (GMP), which means that the additive in question is self-limiting in food products including yoghurt with respect to technological aspects, sensory properties or for other reasons. Thus, GMP also means that the amount of the substance added to food products during the manufacturing stages shall not exceed the amount required to accomplish the purpose for which the additive is permitted to be added to food (FAO/WHO, 1990). These compounds are also used during the manufacture of flavoured (set or stirred), drinking, frozen and, possibly, dried yoghurt.

Table 2.22 Some popular varieties of yoghurt and an indication of the additives (nature-identical and synthetic) that can enhance the flavour of a fruit base^a

Retail flavour	Compounds naturally present		Important synthetic compounds
	Character-impact compound ^b	Important contributory flavour compounds	
Apricot		γ -Decalactone γ -Octalactone Linalool 3-Methylbutyric acid β -Ionone	γ -Undecalactone
Banana	3-Methylbutyl acetate	Pentyl acetate Pentyl propionate Eugenol	
Bilberry		Ethyl 2- and 3-methylbutyrate Ethyl 3-hydroxy-3-methylbutyrate <i>trans</i> -2-Hexenal	
Blackcurrant		Methyl butyrate Ethyl butyrate 1,8-Cineole Diacetyl	<i>trans</i> - and <i>cis</i> - <i>p</i> -Methane-8-thiol-3-one
Grape, Concord Grapefruit	Methyl anthranilate Nootkatone 1- <i>p</i> -Menthene-8-thiol	Limonene Decanal Methyl butyrate Ethyl butyrate Ethyl acetate Acetaldehyde	
Lemon Orange	Citral	Ethyl butyrate Ethyl 2-methylbutyrate Linalool Octanal (+)-Limonene Acetaldehyde β -Sinensal	15 compounds
Melon (honeydew)	<i>cis</i> -6-Nonenyl acetate	Ethyl 2-methylbutyrate	
Melon (musk)	<i>cis</i> -6-Nonenal	2-Methylbutyl acetate Dimethyl disulfide	
Peach	γ -Decalactone	γ -Octalactone δ -Decalactone γ -Dodecalactone Linalool	γ -Undecalactone
Pear	Methyl and ethyl <i>trans</i> -2, <i>cis</i> -4-decadienoate	Hexyl acetate	
Pineapple		Furaneol Methyl hexanoate Methyl 2-methylbutyrate Methyl and ethyl 3-(methylthio)-propionate	Allyl hexanoate

Table 2.22 *Continued*

Retail flavour	Compounds naturally present		Important synthetic compounds
	Character-impact compound ^b	Important contributory flavour compounds	
Plum		Ethyl nonanoate γ -Decalactone Benzaldehyde Linalool Methyl cinnamate	
Raspberry	1- <i>p</i> -Hydroxyphenyl-3-butanone	<i>cis</i> -3-Hexenol Damascenone α - and β -Ionone	
Strawberry		Mesifuran Furaneol Methyl and ethyl hexanoate Methyl and ethyl butyrate <i>trans</i> -2-Hexenal	Ethyl 3-methyl-3-phenylglycidate
Tangerine		Methyl <i>N</i> -methylantranilate Thymol α -Sinensal Acetaldehyde	

^a Concentration is an important variable. Flavour houses are very skilled in providing concentrates of approved components in the appropriate proportions in an appropriate base. Whether and which compounds may be added differs by country, and is usually legally controlled. ^b A character-impact compound is one where the odour by itself is already strongly characteristic of the named food.

Data compiled from Nursten (1977), Buttery (1981), Maarse (1991) and Winterhalter and Schreier (1993).

Different food products, including alcoholic drinks, have been used to flavour yoghurt and some examples of these are:

- Sweet products (honey, maple syrup, butterscotch),
- Nuts (coconut, hazel, brazil, walnut),
- Cereals (muesli),
- Vegetables (cucumber, tomato, celery),
- Miscellaneous (coffee, mocca, spices, paprika, vanilla).

Flavour is an important aspect of food quality and it is caused by chemicals in food, possibly arising during processing, interactions between chemical components and/or the activity of the starter cultures and their enzymes. The latter will be discussed in Chapter 7, but the following sources are recommended for further reading regarding the science of flavours (Birch and Lindley, 1986; Heath and Reineccius, 1986; Bauer *et al.*, 1990; Acree and Teranishi, 1993; Ashurst, 1994). Nevertheless, some specific compounds have been suggested as flavour additives for yoghurt: (a) odourants and/or flavouring compositions containing a substituted tetralin or indan to modify or enhance berry flavours; the recommended rate of addition was 0.1–2.0 $\mu\text{g g}^{-1}$ of flavoured yoghurt (Gonzenbach and Ochsner, 1983), (b) the use of humulon, which is obtained from hops, as a flavour enhancer in yoghurt about 10 $\mu\text{g g}^{-1}$; this compound tended to suppress the sweetness of the fruit slightly whilst the flavour was intensified (Klusters and Paul, 1987) and (c) the use of unusual

Table 2.23 Permitted food colouring matter arising exclusively from flavouring substances as a result of carry-over

Name of colour	Maximum level (mg kg ⁻¹)
Indigotine	6
Brilliant black PN	12
Sunset yellow FCF	12
Tartrazine	18
Cochineal	20
Carminic acid	20
Erythrosine	27
Red 2G	30
Ponceau	48
Caramel ^a	150
Brilliant blue FCF	200

^a Ammonia or ammonia sulphite process.

Data compiled from FAO/WHO (1990).

flavours in yoghurt such as herbs (Anon., 1993a) or geranium, elderflower, apple blossom and rosehip (Winwood, 1987).

2.12.3 Colouring matter

Colour is added to fruit and flavoured yoghurts to make the products more attractive (Pasch *et al.*, 1975; Ulberth *et al.*, 1993). The active agents may be naturally derived, nature identical, caramel or artificial (Collins and Timberlake, 1993). The list of colours which may be used as food additives differs from one country to another, but it should be noted that the colouring agents permitted in one country may not be identical to those allowed in another. However, the FAO/WHO (1990) have offered some guidance about which colour compounds should be permitted and at what concentrations in yoghurt, assuming that the agents come entirely from the fruit/flavouring ingredients (see Table 2.23). A list of natural colours that can be used in food including the E-number has been provided (Anon., 1993d), whilst Hod (1995) has listed kosher food colouring ingredients.

Fourteen permitted food colourants in South Africa have been evaluated in yoghurt making and were added at 75% of the permitted level (Venter *et al.*, 1988); heating and fermentation of the milk were identified as causing colour bleaching. Otte (1988) reported that by increasing the fat content in the milk base, the colour intensity decreased whilst the colour shade and saturation were only slightly affected. Flavonoids from sandalwood (*Pterocarpus santalinus*) and roselle (*Hibiscus sabdariffa*) have been used to intensify natural colours of fruit purees for yoghurt (Labatut, 1989).

2.13 Packaging

2.13.1 Introduction

Packaging is an important step during the production of yoghurt and Paine (1967) has defined the objective of packaging food as: “Packaging is a means of ensuring

the safe delivery of product to the ultimate consumer in sound condition at minimum overall cost.”

In general, the specifications of any food packaging material should include information for the following:

- Toxicity of the materials,
- Levels of contamination,
- Moisture resistance and/or permeability to water vapour,
- Gas permeability for N₂, CO₂ and O₂ (the former gases are important in modified atmosphere packaging),
- Permeability to volatile flavour and aroma compounds and/or chemicals in the environment,
- Transparency to visible or UV light,
- Permeability to dirt and/or to micro-organisms, and
- Migration of molecules from the packaging material to the product.

It is evident that most, if not all, of the above mentioned specifications for packaging material are applicable to yoghurt packaging. However, as can be observed from the subsequent sections, there are many different types of packaging materials and some selected publications on the theory and practice of food packaging have been reported by Paine (1969), Sacharow and Griffin (1970), Paine and Paine (1983), Griffin *et al.* (1980), Peleg (1985), Mathlouthi (1986), Briston (1989), Jenkins and Harrington (1991), Stöllman *et al.* (1994) and Soroka (1995). Periodically the International Dairy Federation publishes monographs updating the technical information available on the packaging of milk and milk products and specific aspects dealing with fermented milks have been reported by Fluckiger (1976, 1980, 1982) and Odet (1984, 1988, 1995).

2.13.2 Functions of packages

If yoghurt is to reach the consumer in a sound condition, the packaging material will play an important role, and the retail package should be designed to meet the requirements, to provide protection, to be easy to handle, to provide a vehicle for a message and so on.

2.13.2.1 Provide protection

Yoghurt is a highly perishable product and the purpose of the container is to protect it from the environment, that is: (a) dirt or other foreign bodies, (b) micro-organisms (bacteria, yeast and moulds) which can affect the keeping quality of yoghurt, (c) gases (e.g. oxygen) which can help the yeasts and moulds to grow and spoil the product, and (d) light which may cause discoloration of fruit/flavoured yoghurts or possibly oxidation of the fat.

Product protection also seeks to avoid spillage, pilferage or loss by evaporation. The latter aspect is doubly important, since loss of moisture can not only affect the chemical composition of the product, but may also lead to deviations from the declared weight on the package and possible problems with the weights and measures authorities. In addition, the package must prevent the loss of flavour volatiles or the absorption of undesirable odours. However, a detailed study by Bosset and Fluckiger (1986a, b, 1987) and Bosset *et al.* (1986a, b, 1995) evaluated the impact of environmental aspects, such as light and temperature on the quality of yoghurt

packaged in different containers (e.g. glass jars (coloured and uncoloured), polystyrene cups (transparent coloured or uncoloured) and non-transparent overwrapped with a cardboard sleeve), and suggested the following:

- The protective effects differed with the type of packaging material used and protection from light was identified as affecting the quality of the product more than protection from oxygen, though these factors can have synergistic effects; however the rate of O₂ permeability across the packaging material should be very low for long life or pasteurised yoghurt.
- Transparent uncoloured glass jars or polystyrene cups provided least light protection; the latter type of package also had high gas permeability.
- Depending how the containers were stored (on its side, upright or upside down), the aluminium foil cover and polyethylene lids gave less effective protection of the product.
- Less damage to the product could be achieved during storage by applying the following protective measures. In the absence of aseptic facilities during production, filling and storage at 8°C should not exceed 16–18 days. Use of fluorescent lighting caused less damage, e.g. Philips TL82.
- It was noted also that the sensitivity of the analytical methods used to measure the degree of photodegradation in the product varied.

2.13.2.2 *Ease of handling*

Yoghurt and related products usually exist in the form of viscous liquids. The retail container must provide a convenient means of handling the product in the factory, during storage and transport, and throughout the sale period in supermarkets and shops.

2.13.2.3 *Provide a message*

The printing and other graphic work on the exterior of the package will serve to provide the product with a “brand image” and/or display a message to persuade a potential buyer to purchase, and will contain the information proposed in the guidelines for food labelling such as:

- identity of the product,
- name and address of the manufacturer,
- approximate chemical composition or nutritional data of the product, or the ingredients listed in descending order by weight,
- best-before-date,
- possible suggestions of recipes or other instructions for use.

2.13.2.4 *Miscellaneous functions*

In general a packaging material which is in direct contact with a foodstuff must be non-toxic and no chemical reactions should take place between the material and the food product (refer to Crosby, 1981; Jensen, 1972; and Section 2.13.1 for further information). For these reasons, plastics are widely used in the dairy industry, and due to the acidic nature of yoghurt, aluminium foil is used for lids, unless plastic “push-on” lids are more suitable.

It is against this general background that the following approaches to marketing yoghurt have evolved.

2.13.3 Types of packaging materials

Packaging materials for yoghurt are basically divided into two main categories: in the first instance, the unit container is the vessel which comes into actual contact with the yoghurt and the specifications mentioned above regarding the “ideal” package are applicable to such containers, and in the second instance, the outer or shipping container does not come into contact with the yoghurt, but is used to facilitate handling and dispensing of the unit containers along the retail chain.

Different types of unit container are available on the market, and these packs may be classified into three main types depending on the physical strength of the container.

2.13.3.1 Rigid unit containers

Glass bottles are still used in some countries, for example, France and Eastern Europe and some parts of the Middle East to package yoghurt (Fluckiger, 1980), and although glass is an excellent packaging material, its use is limited by the high cost of manufacture and the current market trend in favour of “single-trip” containers. Nevertheless, the glass bottle was very popular and, even today, wide mouth glass bottles are the most attractive form of packaging for flavoured yoghurts; closure is by a metal “pull-ring” or screw-on metal cap. A closure system involving heat sealing aluminium foil laminates onto glass bottles is available in different markets.

The system of recycling or returning the empties on a voluntary basis could affect the use of glass bottles and some studies have been carried out in Switzerland and Germany (Anon., 1983b; Berndt, 1984; Regez, 1984). It may be that the increasing pressure to improve the environment and to educate the consumer will increase use of returnable glass jars for yoghurt packaging.

Earthenware vessels are produced from clay and the part of the container which comes in contact with the yoghurt is normally glazed. They are returnable and are used in the Middle East and India to package set yoghurt and dahi, respectively. During the incubation period the pots are left uncovered so that a crust is formed on the surface and, before the cooling stage, the pots are covered with parchment held firmly in position using a rubber band. These containers are not widely used due to problems of achieving a high standard of hygiene and the cost of manufacture. Thus, Singh (1978) evaluated the microflora of earthenware pots used for dahi and reported that high total counts, as well as coliforms, *Staphylococcus* spp. and yeast and moulds, were normally present. Improvements in the microbiological standards of these pots could be achieved if the pots were immersed in boiling water for at least 2 min or water containing $250\text{--}500\mu\text{g g}^{-1}$ chlorine. However, the clay used to manufacture this earthenware may contain $30\text{--}100\mu\text{g g}^{-1}$ of lead and, as a consequence, the lead content of set-type dahi made in these pots was $1.743\mu\text{g g}^{-1}$. This level of lead is still below the permitted limit ($7\mu\text{g g}^{-1}$) proposed by the Food and Drug Administration in the US (Nagaraja and Vishweshwaraiah, 1986), but it is another factor that operates against the use of earthenware.

Other rigid containers that are recommended for the packaging of some types of yoghurt-based products, for example, dried yoghurt, are metal cans or aluminium foil laminated pouches. The keeping quality of the product is improved by gas flushing (nitrogen or carbon dioxide), nitrogen being more widely used. These metal containers are similar to those used for packaging of whole milk powder. Rigid,

semi-rigid or flexible plastic containers could also be used (see later), as could composite containers coated internally with a layer of plastic.

2.13.3.2 *Semi-rigid unit containers*

These types of container are normally manufactured from plastics and some technical properties of the different types of plastic material (i.e. water vapour transmission rate and gas transmission rate of O₂, CO₂ and N₂) that can be used for the manufacture of containers for yoghurt have been reported by Kumar (1989), Cuq *et al.* (1995) and Guilbert and Gontard (1995). The actual plastic materials (i.e. the polymers) are relatively inert, but the chemicals and monomers used during the fabrication stages can be deposited in the finished material. Although such compounds may be harmless *per se*, they can react with the food and give rise to off-flavours, and hence great care has to be exercised to ensure that such compounds are absent.

In the case of yoghurt, the container must be acid resistant, prevent the loss of flavour volatiles and be impermeable to oxygen, since the presence of the latter can encourage yeast and moulds to grow. Examples of materials which could be used for the manufacture of the yoghurt containers are: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyvinylidene chloride (PVDC). In the United Kingdom, the majority of the containers are manufactured from PS, although the use of PP is becoming popular. However, irrespective of the material, the containers can be either rigid, semi-rigid or flexible, and while the former categories are normally used to package set and stirred yoghurt, concentrated yoghurt and/or frozen yoghurt, the flexible type (i.e. film – see later) can only be used to package dry yoghurt-based products.

The finished containers are referred to as cartons, tubs or cups and can be manufactured in any shape or design that appears to possess consumer appeal; some typical examples are illustrated in Fig. 2.18. Basically, there are two different techniques that can be used for the manufacture of plastic cups. Firstly, the injection moulding process in which the material is softened in a heated cylinder prior to injection under high pressure into a cooler/mould where it hardens. After the cup is formed, it is ejected from the mould. This type of container is characterised by having a relatively thick wall, that is, it is a rigid cup (Astrom, 1989; de Groof, 1993). These preformed cups are then delivered to the dairy nested in rows inside a thin PE bag (i.e. of 25 µm thickness). Usually the bags are sealed to prevent the ingress of dust or other contaminants and are overwrapped with a fibreboard box to ensure safe transit and prevent crushing. At the dairy the rows of cups are fitted onto the filler and the process of packaging is then referred to as a fill/seal operation. Second is the thermoforming process, in which the plastic material is delivered to the dairy in the form of a continuous roll, one end of which is fed into the first section of the yoghurt filling/packaging machine. The sheet of plastic is heat softened and formed into or around a mould, so that the unit container is formed immediately prior to filling with the yoghurt. This system of packaging is referred to as a form/fill/seal operation. In the thermoforming process, the yoghurt cups have a relatively lower wall thickness than those produced by the injection moulding system, and the containers could, therefore, be classified as semi-rigid. Incidentally, the reels must be delivered to the dairy well overwrapped so that they do not get damaged in transit or storage.

Irrespective of what types of cup are used, closure of the container is usually achieved using aluminium foil (i.e. capping/crimping or heat-sealing) or plastic



Fig. 2.18 Illustrations of some containers for the packaging of yoghurt

Top row: Illustrates multipack containers and different packs for drinking yoghurt (e.g. rigid, plastic bottles and cups, and a ring-pull metal can). Middle row: Yoghurt containers consist of glass jars (left hand side) and twin chamber packs (right hand side). Bottom row: Laminated paper board cartons (Pure-Pak and Tetra Brik); some have recloseable lids or special hole for a straw (left hand side). Containers on the right hand side illustrate thermoformed and preformed plastic containers.

(press-on lids or heat sealing). Heat-sealed caps are more popular, since the cups are then watertight and subsequent contamination and seepage are prevented. Aluminium foil is used because its permeability to gases and odours is negligible, and, in addition, it is greaseproof, opaque, “brilliant” in appearance and can be easily decorated. Because of the acidic nature of yoghurt, it is recommended that the foil should be lacquered to prevent corrosion and, to provide cohesion during heat sealing, the foil should be coated with PE, ethylene vinyl acetate copolymer (EVA), PS or PVC (see later for further detail). Heat-sealed plastic lids are sometimes used.

Plastic packaging materials are also used in the yoghurt industry to provide so-called fresh crunch products for the consumer. An example of one such innovation was reported by Colangelo (1980) and Anon. (1983c, 1991b) in which the yoghurt was packaged in what was referred to as a “piggy-back” configuration. In this system the flavoured yoghurt is filled and sealed into a plastic cup. Either on the same machine, or on a different unit, nuts, raisins and carob chips (known as granola) are filled into another transparent cup which is also heat sealed. Then the yoghurt tub and the cup containing the granola slot together so that the latter container completely covers the yoghurt tub prior to the two packages being heat-sealed or crimped together. In theory, the freshness of the fruit/nut mixture is retained until the consumer mixes the two components together just before consumption, but there is little evidence to date that the additional labour is really justified.

Another type of semi-rigid cup (i.e. 2–4 cm in depth) has been patented in France (Verdier, 1987) and is suitable for packaging yoghurt to enable consumers to eat the yoghurt using their tongues without the aid of a spoon. Two pots can be attached

together to provide an adequate portion of the product, but this type of package has not been used yet by the industry.

As a saving on the cost of rigid plastic beakers, a thin polystyrene beaker was suggested, surrounded by an envelope of recycled paper (Poldervaart, 1994). This type of packaging is known as the K-3 system and allows easy separation of the paper from the plastic by flattening the container when empty. Nevertheless, the current interest of plastic manufacturers is to replace PS with PP because (a) there are cost savings (Recaldin, 1990), (b) there are residual effects of the styrene monomer in the product (see Section 2.13.5) which could affect the flavour and (c) burning PS at $<1200^{\circ}\text{C}$ yields large quantities of soot that pose an environmental problem (Løkkeberg, 1993). However, for the purposes of recycling mixtures of plastic consisting of PS and PP, the two plastics can be separated, after washing to remove organic material and grinding, on the basis of specific gravity, using a hydro-cyclone or swim-sink process (Wirths, 1991).

2.13.3.3 Flexible unit containers

Flexible unit containers are either in the form of plastic sachets or paper cartons. The former type are made from laminates, (e.g. PE/aluminium foil/PE or PE/paper/aluminium foil/PE) and are only used to package dehydrated yoghurt. The most popular method of filling is the form-fill-seal approach. The container must be impermeable to gases and water vapour.

Paperboard cartons became a popular container for dairy products in the 1950s with the introduction of the waxed cartons. These containers were used in the past for the packaging of yoghurt, but their popularity has diminished in favour of plastic cups and/or laminated paper cartons. One disadvantage associated with the waxed carton is its tendency to leak and despite improvements in manufacture (i.e. the application of a multilayer coat of wax and EVA copolymer), their use as yoghurt containers in the United Kingdom has remained limited.

However, the use of cartons to package liquid milk is widely practised in North America, Europe and the United Kingdom and such containers could be easily used to package yoghurt (Fig. 2.18). Two types of carton are normally available, a simple type where ordinary paper board is coated on both sides with a plastic material, (e.g. PE) and a multilayer type which consists of the following layers: PE/paper board/aluminium foil/PE. The latter type of carton is normally used for packaging UHT milk, since the aluminium foil layer not only renders the carton impermeable, but also helps to improve the rigidity of the container.

Depending on the exact system employed, paperboard cartons are delivered to the dairy either as collapsed preformed cartons (e.g. the Pure Pak, Elopak or Tetra Rex methods) or in the form of a reel (Tetra Brik). The sequence of packaging followed for preformed cartons is:

- A bank of collapsed cartons is fed into a special sleeve of the filling machine.
- A single carton is automatically removed from the sleeve, opened and the bottom is sealed.
- The carton is filled with yoghurt and the top sealed.
- The packaged product is ready for dispatch.

Alternatively, the cartons can be formed from a reel using the technique of form-fill-seal and an illustration of the sequences involved in the formation of one such

container, prior to filling it with yoghurt, is shown in Chapter 3, Section 3.3.11. One common feature of these packaging systems (e.g. Pure Pak, Tetra Rex or Elopak) is that the carton has a gable end. In some instances this gable structure may prove to be useful for pouring the product, but one disadvantage is the large storage area required compared with that needed for yoghurt packed in flat-top cartons. However, the recent development of the “flat top” Tetra Rex and Pure Pak cartons, which are relatively square in shape, combines the desirable features of a gable (i.e. excellent pouring characteristics) with efficient utilisation of the space in refrigerated cabinets.

Improvements in packaging materials for cartons used for fermented milk products, including the effect on the quality of the product, are (a) the CO₂ concentration of kefir and similar cultured milks packaged in double-layered PE was reduced by 75% and whey drainage occurred, whilst with aluminium foil-lined cartons, the CO₂ concentration was reduced only slightly and the flavour and stability of the products were significantly improved (Anon., 1986c; Castberg *et al.*, 1986; Gjengedal and Oterholm, 1988), (b) the introduction of a specially designed filling valve into a gable-top carton using a Cherry Burrell QL-9 machine makes it possible to package a drinkable type of yoghurt (Anon., 1986d), (c) Ensobarr® is a newly developed paper board gable-top carton from Finland which is suitable for packaging yoghurt; the aluminium foil layer has been replaced by a Chemi Thermo Mechanical® pulp, which provides a given stiffness using less weight of paperboard, and has better folding and recycling properties (Holmström, 1996), and (d) the provision of a straw for 250 g Pure Pak, or a screw cap for 1 l cartons increased the packaging options for drinking yoghurt (Schlicht, 1996).

Finally, the laminated paper cup is used in the U.S. and some other countries where the cups are preformed and delivered to the dairy nested in cardboard boxes. These cups are sealed using press-on lids or possibly heat-sealed foil lids (Anon., 1984b, c).

The size of the above containers is divided into two main groups, “single serve” cartons, with content ranges from 150 to 200 ml (in some cases it may be less), and “family size” cartons where the capacity of the container ranges from 250 to 1000 ml (Herner, 1988). In the latter sizes, press-on lids are also provided, since not all the yoghurt may be consumed at the same time and it is necessary to provide, for reasons of hygiene, a lid which can be reclosed. Incidentally, a type of intermediate container which is becoming very popular is the special purpose multipack (see Fig. 2.18), where four, six or more yoghurt cartons are packaged together. These multipacks were introduced into the market in the 1970s (Lang, 1972; Chaussadas, 1986; Anon., 1989; Keck, 1983, 1991a; Hilliam, 1992; Hartman, 1995), and are sometimes used when launching a new fruit yoghurt onto the market, or alternatively they are used as a family pack. A similar type of family pack is now widely produced on form-fill-seal machines (i.e. thermoformed), where four or more yoghurt cartons (each pair is a different flavour) are formed as one composite unit. Over the past decade a twin chamber or tub pack has been introduced and in this pack natural yoghurt is filled into the larger compartment whilst the fruit flavour is packaged in the smaller chamber. The main advantage of this pack is that the yoghurt manufacturer need not stop and clean the equipment when changing from one product flavour to another (Ackermann and Guays, 1984; Zott, 1989). The normal practice is to start by filling the light coloured fruits and progressively changing to the darker types. Although expensive, this system of packaging is popular with

the consumer who can mix the fruit with the yoghurt prior to consumption in a quantity to suit his/her palate.

It is evident that a wide range of packaging containers are being used in the yoghurt industry and the ultimate choice could be influenced by the following considerations:

- Cost per unit container, speed of filling and cost of packaging machine,
- Nature of the yoghurt products (e.g. liquid, viscous, concentrated or powder),
- Provision of product protection during storage, distribution and retailing,
- Capacity of the unit container,
- Returnable or non-returnable package and, in the case of the former type, whether the container can be cleaned and sanitised,
- Requirements for a specific duration of shelf life, including the barrier properties (e.g. O₂ and light permeability) of the material,
- Marketing concepts and consumer acceptability (Odet, 1988).

One aspect, which has always been debated in the dairy industry, is the scenario regarding glass versus non-returnable containers including cartons and plastic cups or bottles. Some reports have been published by Anon. (1983b, 1988, 1994b), Bojkow (1986), Keck (1991b), Robinson (1991) and Thalmann and Schmid (1996). Cost and environmental aspects are the major factors which have to be considered before choosing any one type of packaging material, but according to Robinson (1991), the solution of waste problems is difficult and, in the case of plastics, may have serious consequences in the future.

2.13.4 Comparative studies on the permeability of different yoghurt packages

As mentioned earlier, the work of Bosset and Fluckiger (1986a, b, 1987) and Bosset *et al.* (1986a, b) has highlighted the effect of light and O₂ on the quality of yoghurt held in a wide range of containers. However, other studies which have shown a direct correlation between the permeability of the packaging material and the quality of yoghurt can be summarised as follows:

- The O₂ permeability (kPaday⁻¹) through a Pure Pak carton (i.e. PE/paper board/PE) was 0.77 mg and 1.79 mg at 7°C and 25°C, respectively; these permeability values were about half the theoretical values calculated for the O₂ permeability of PE layers of 0.03 mm and 0.015 mm, respectively (Langeveld *et al.*, 1984).
- Cultured buttermilk packaged in high density PE bottles was stored at 1°C and exposed to white fluorescent lighting for 96 hour, and these conditions induced an off-flavour and reduced the riboflavin concentration by 0.3 µg ml⁻¹. A taste panel could not identify the light-induced off-flavour in the experimental samples (Hoskin, 1989); Bosset *et al.* (1995) also confirmed that yoghurt is a light-sensitive product.
- Yeast and mould counts (>10 colony forming units (cfu) g⁻¹) were detected in 30 out of 60 containers of yoghurt in preformed containers, whilst none of these organisms were detected in the same yoghurt packaged in form-fill-seal containers (Jordano, 1987); this may reflect differences in O₂ permeability and/or the sterility of the container before filling (McKay, 1992).
- Brown glass bottles prevented photo-oxidation of yoghurt (natural, strawberry, chocolate or mocca), whilst only natural yoghurt was susceptible to light when

packaged in transparent PS containers (Dieffenbacher and Trisconi, 1989); a marked decrease in the green and yellow hues due to loss of riboflavin was noted (Desarzens, 1989) and pentanal was identified as the carbonyl compound produced during photo-oxidation (Daget, 1989).

2.13.5 Migration of monomers and other compounds

In the U.K., according to the Ministry of Agriculture, Fisheries and Food (MAFF, 1983, 1987), there were until 1974 insufficient toxicological data available on styrene to assess the long term safety of food in contact with it. Since then there have been numerous analytical studies and the results of surveys of styrene levels in yoghurt in different countries are shown in Table 2.24. It is evident, however, that the residual styrene in yoghurt is well below the tolerable daily intake ($0.6\mu\text{g g}^{-1}$) proposed by the EU Scientific Committee for Food (Hammarling *et al.*, 1995).

Factors that can influence the migration of many compounds from packaging materials, including PS and PP, include product filling temperature, fat and moisture contents and pH (Thomsen and Stena, 1987). However, the migration of monomers does not affect the colour of yoghurt and the rate of migration after 2 days at 45°C was not more than 1.1 mg dm^{-2} (Macias Matos *et al.*, 1988). Nevertheless, caution is essential and three out of 20 yoghurt pots made in Cuba exceeded the migration limits set by the Hungarian Standards (Garcia Melian *et al.*, 1988). Both yakult (a Japanese fermented beverage) and yogo (a drinking yoghurt) sampled in Hong Kong contained no styrene monomers, despite the fact that the styrene content of the yogo plastic cup was about $150\mu\text{g g}^{-1}$ (Lau *et al.*, 1995). Another monomer that has been detected in yoghurt is ethylbenzene at a level ranged between 2 and $4\mu\text{g kg}^{-1}$ (Ehret-Henry *et al.*, 1994).

Cultured skimmed milk from three factories packed in Tetra Pak and Elopak cartons contained different levels of aluminium after storage at 4°C for 20 and 40 day, respectively; the results suggested a slight increase ($8\text{--}18\mu\text{g kg}^{-1}$), while some samples showed a reduction ($3\text{--}42\mu\text{g kg}^{-1}$) in the aluminium content after storage (Eklund and Brenne, 1990). These results may indicate that the increase or decrease in aluminium content was influenced by the type of cartons or milk utilised for processing, although the level was satisfactory according to Eklund and Brenne. Further

Table 2.24 Styrene content in yoghurt made in different countries

Country	Number of samples	Fat ^a	Residual styrene (range)
U.K.	34	NR	$<1\text{--}200\mu\text{g kg}^{-1}$
Chile	16	NR	$0.08\text{--}0.19\mu\text{g kg}^{-1}$
Holland	8	0.1–0.5	$3\text{--}4\mu\text{g kg}^{-1}$
	16	1.5	$5\text{--}11\mu\text{g kg}^{-1}$
	16	3–3.5	$2\text{--}5\mu\text{g kg}^{-1}$
Sweden	11	NR	$\sim 0.01\text{ mg kg}^{-1}$
France	4	0	$10\text{--}12\mu\text{g kg}^{-1}$
	6	3.5	$8\text{--}11\mu\text{g kg}^{-1}$

^a Fat content expressed as $\text{g } 100\text{ g}^{-1}$.

NR, Not reported.

Data compiled from MAFF (1983), Gilbert and Startin (1983), Carmi and Santa Maria (1984), Linssen *et al.* (1993), Ehret-Henry *et al.* (1994) and Hammarling *et al.* (1995).

Table 2.25 Taste recognition threshold concentrations of styrene in yoghurt

Product	Fat (g 100 g ⁻¹)	TRTC (µg g ⁻¹)
Yoghurt		
Natural	0.1	36
Natural	1.5	99
Natural	3.0	171
Yoghurt drinks		
Natural	0.1	82
Strawberry	0.1	92
Peach	0.1	94

migration studies from paperboard packaging material have been detailed by Castle *et al.* (1997a, b), whilst Linssen *et al.* (1992) reported that volatile compounds (i.e. >8 carbon atoms) and highly branched components in artificially flavoured drinking yoghurt tended to be absorbed by PE bottles. Incidentally, these PE bottles were composed of three layers: a PE layer with 2% carbon sandwiched between two PE layers with 5% TiO₂ to create white inner and outer layers.

Although the styrene monomer can only, in part, be removed from the polymer by extrusion of the packaging material (Linssen *et al.*, 1995), the level of residual monomer can cause off-flavours in the product. Thus, flavour threshold values of such monomers are important parameters with respect to detecting off-flavour perception in food products. Jensen (1972) reported the following flavour threshold for monomers from PS (µg g⁻¹) in yoghurt: 0.2 styrene, 0.9 ethylbenzene, 7.0 *o*-xylene and 1.0 cumene. However, the taste recognition threshold concentration (TRTC) of styrene in yoghurt is influenced by the presence of sugar and flavouring material and the level of fat (Table 2.25; Linssen *et al.*, 1993, 1995).

2.13.6 Tamper-evident packaging

Since the 1980s, the general consensus in the food industry has been in favour of tamper-evident packaging that enables food retailers and consumers to identify opened packages easily. This approach has become universal in the yoghurt industry, because the older systems of closure (e.g. snap-on plastic lids or crimped foil caps on glass jars) are not acceptable safeguards of public safety.

Many different types of tamper-evident packs have been developed for the food industry and the systems available for yoghurt include the following:

- Heat sealing of foil laminates to a plastic container should ensure a secure seal so that tampering with the container shows as visible damage to the foil.
- Sealed cartons (e.g. Pure Pak or Elopak) have flaps that are securely sealed and tampering shows as visible damage.
- Shrink-wrap film around yoghurt pots sealed with press-on plastic lids or around a set of pots provides another method of packaging that is tamper-evident.
- Hot filling of yoghurt causes the foil laminate heat-sealed lids to adopt a concave shape after cooling; opening the plastic cup causes the lids to change shape and a similar effect occurs with metal caps if yoghurt is packed in glass jars.

- Heat sealing of press-on plastic lids (see Johansen and Buer, 1991) is a newly developed tamper-evident system.
- Breakage of the aluminium ring-pull, which is sometimes used to seal glass bottles, indicates tampering.
- In some instances, a narrow paper strip is sealed over the metal cap used for glass jars and the seal must be broken to open the jar.
- The use of security closures, such as “pull-up” plastic strips or pilferage proof screw caps on wide mouth plastic jars or rigid plastic bottles and cartons, respectively, provide alternative tamper-evident systems which have been used in the yoghurt industry; such systems are sometimes known as breakable caps.
- Tear away closure systems include a “pull-tab” attached to a tamper-evident device consisting of horizontal and vertical ribs; these devices are ruptured easily (i.e. removal of the central portion of the lid) due to force applied on pull-tab (Anon., 1995b).

It is evident, however, that different tampering-evident systems can be used with all the yoghurt packaging containers mentioned in Section 2.13.3 and although these systems can increase the cost of production, product safety can satisfy consumer concerns. More details of tamper-evident systems for foods, including fermented milk products, have been reported by Herner (1987c), MacDonald and Cox (1988) and Freeman (1992).

2.13.7 Aluminium foil lids

Aluminium foil is widely used to seal yoghurt containers (e.g. plastic cups) and due to the acidic nature of yoghurt and the necessity of heat sealing, the aluminium foil is normally coated with a layer of plastic. If the preformed type of plastic cup is used, the aluminium foil lids are usually precut and around 2500–3000 lids are packed into a special magazine to minimise mechanical damage. The diameter of these lids is <100mm, and they usually have a pull-tab for easy opening.

The gauge of the foil is around 40µm, and each lid is normally embossed. The embossing pattern can be varied to suit the customer's requirements and the impression can be up to 100µm in depth. The embossing is essential to facilitate easy pick-up of single lids from the magazine assembly prior to placing over the filled cup and heat sealing.

For yoghurt packaged using the form-fill-seal technique, the aluminium foil is delivered to the dairy as a reel, with the width of the reel being varied in relation to the number of filling heads (abreast) on the packaging machine. The aluminium foil (gauge around 40µm) is similar to that of the precut type, but the embossing process is omitted since it would serve no function.

As can be observed from packaged yoghurt on the market, both types of lid (precut or from a reel) can be printed with different information and attractive designs. The technique of printing could be the flexograph or the gravure; the latter method is normally used where more than five different printing colours are required. The reverse side of the lids is coated with heat-sealable material and the thickness of the laminate ranges between 6 and 10gm⁻². The variation in the thickness of the lacquer is directly dependent on the type of heat-sealing material used and, for example, 6–8gm⁻² of EVA is applied to foil intended for heat sealing to polystyrene or polypropylene. For the latter type of plastic cup, a modified version

of EVA is used, that is, it has a stronger solvent intended for higher temperature sealing purposes. On the printed side of the aluminium foil, the EVA lacquer is coated with a layer of high heat varnish in order to protect the graphic design during the heat-sealing stage at the dairy (J.R. Englehart, personal communication). Elms (1989) has reviewed the latest applications of ethylene acrylic acid (EAA) as a sealant for flexible packaging.

Some further types of lids for plastic pots may include: (a) a breathing membrane which consists of a three-ply lidding system that is suitable for packaging kefir in order to minimise the pressure buildup (due to considerable CO₂ production) that would ultimately lead to heavy bulging of the container (Fluckiger, 1986), (b) a specially designed pot and metal foil sealing system for packaging hot yoghurt that eliminates the vacuum generated inside the pot during the cooling of the product (Padovani, 1987), (c) a sealing system for pots containing drinking yoghurt that consists of a lightweight paper and thin foil laminate with a weakened zone for insertion of a straw (Huet, 1986), (d) a cap with a peripheral rim that is strong and easily peeled off the container without undergoing distortion (Kretz, 1987), (e) a new type of foil, which has been developed in Hungary, consisting of a laminate of PP/PE (Stark, 1986), (f) paperboard flexible foil laminates interacting with lactic acid were observed not to adhere during storage (Olafsson and Hildingson, 1995); one reason for this could be the polarity and chemical structure of the acid, and (g) a welded seal plastic lid which can be replaced on a partially empty container once the seal has been broken; this development makes the lid tamper-evident (Johansen and Buer, 1991).

2.13.8 Sterilisation of packaging materials

Sterilisation of packaging materials (i.e. plastic cups and lids, foil laminates or paperboard cartons) in the yoghurt industry ensures that possible post-production contamination of the product is minimised and meets the criteria required when using an aseptic processing system. These criteria include that the yoghurt must be sterile, the packaging container and/or materials in which the product is packed must be sterile and the environment/chamber where the sterile product and containers are brought together must be also sterile (Ito and Stevenson, 1984). Illustrations and descriptions of aseptic yoghurt machines will be given in detail in Chapter 3. Little published data are available on the microbiological quality of packaging containers; however, Asperger (1983) reported that the total count (i.e. mainly sporeformers) of plastic cups was <10 cfu 100 ml⁻¹ rinse and coliforms or moulds were absent. Data on microbial properties of other packaging materials were also reported by Asperger and in view of such information, it is clear that the sterilisation of yoghurt packaging material is necessary during the manufacture of "long-life" yoghurt.

It is safe to assume that form-fill-seal yoghurt plastic cups are commercially sterile due to the high temperature required to make these containers. However, preformed plastic cups and/or collapsed cartons may require sterilisation before filling them with yoghurt in order to minimise postproduction contamination. Astrom (1989) has reviewed the different systems available to sterilise the packaging material for aseptic process and these may include the following methods:

- hydrogen peroxide (H₂O₂) spray, dip or vapour,
- steam,

- hot air impregnated with H_2O_2 ,
- thermoforming containers using form-fill-seal system, perhaps plus H_2O_2 ,
- dry hot air,
- ethylene oxide,
- combination of H_2O_2 plus UV light,
- gama radiation (see also Ito and Stevenson, 1984; Neijssen, 1993).

Radiation and H_2O_2 sterilisation of dairy packaging materials are the most commonly used methods; however, in the latter method, the sterilant is removed by heat and its residue on the surfaces may have delayed its acceptability in some countries. Nevertheless, the effects of H_2O_2 sterilisation on the migration of monomers of PP and PE to the food are negligible (Castle *et al.*, 1995) and the only slight change made to these plastics was a superficial modification of the polymer surface.

The use of UV-C lamps to sterilise yoghurt plastic cups and foil laminates was first reported in the early 1980s in an aseptic Hamba filling machine (E. Möller, personal communication). The filling/closure operations take place in a cabinet which is kept free of contaminants by a stream of sterile air at 30–40°C; this air temperature is recommended to prevent condensation. The components of the filling machine are cleaned and sterilised using cleaning-in-place (CIP). The development of UV-C lamps for Hamba machines was well documented by Möller in a lecture in the Department of Milk and Dairy Industry at Hanover University in Germany (see also Hansen, 1980; Möller, 1982). The intensity of the UV-C lamp is in the range of 100–200 mW cm⁻², and the distance between UV-C lamp and the packaging material is adjusted in such a way that the cups are at a distance of 10.5 cm; the total exposure time is around 7.5 s and three emitters are used to sterilise the entire inner surface of the cup. The aluminium foil lids are exposed for 2 s at a distance of 4 cm. The efficiency of UV-C lamps against different bacterial species is illustrated in Fig. 2.19 and according to Möller (1982), the shelf life of fruit yoghurt packaged in containers sterilised by UV-C lamps was extended to 42 days at 5–7°C.

In some instances yoghurt may be aseptically filled in bags or bag-in-box containers (see Prahlad, 1989). Martin (1982) details the facilities required for such methods of packaging, and these comprised a laminar flow cabinet fitted with air sterilisation, decapping, filling and recapping devices. Along the side wall of the cabinet, an opening provided access through which the prewrapped sterile bags were introduced to be unwrapped, filled and capped. A UV sterilisation system was provided and the method of packaging could be either fully or semi-automated.

2.13.9 Outer or shipping container

These types of packaging material do not come into contact with the yoghurt, but their importance in the industry is to facilitate easy handling and stacking of the cups during storage, transport and display in supermarkets. Different types of container can be used, divided into two groups, returnable and single-trip.

The returnable containers (or crates) are made of metal or rigid plastic, but since the crates require collection, they are not widely used. However, metal crates are popular where set yoghurt is produced in glass bottles and the fermentation process takes place in a water bath. This method of processing is not widely used.

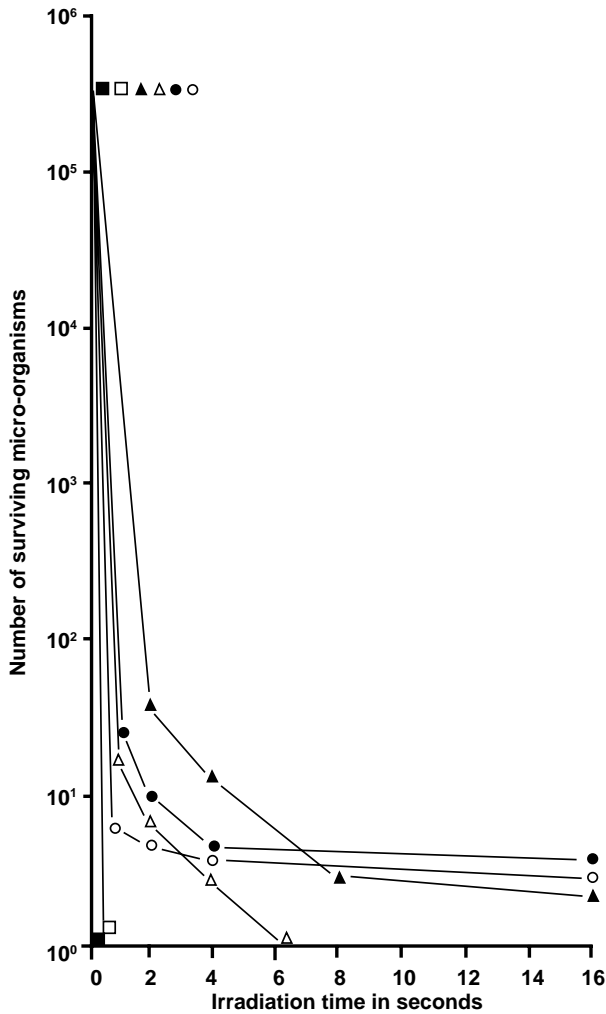


Fig. 2.19 Inactivation of bacteria, yeast and moulds by UV-C radiation

Total microbial load is 5×10^5 cfu ml⁻¹ and test area is 36 cm².

●, *Bacillus stearothermophilus*; ○, *Bacillus subtilis*; ▲, *Aspergillus niger*; △, *Penicillium frequentans*; ■, *Saccharomyces cerevisiae*; □, *Rhodotorula graminis*.

After Möller (1982, personal communication).

Single-trip containers are more widely used than the returnable type and some of the different types available on the market are:

- semi-rigid plastic crates
- nest trays (flexible plastic or any similar material – see Anon., 1980b)
- cardboard trays.

The latter types of tray (or paperboard cartons) can be overwrapped with a heat-shrink material or alternatively the nested trays can be piled on top of each other (4–6 trays high) inside a cardboard box.

The choice of any one particular system and/or type of outer container is governed primarily by such factors as:

- cost,
- degree of mechanisation,
- ease of dispensing and marketing,
- stackability and ease of cold air circulation in the refrigerated store.

This latter aspect is important if the yoghurt is filled at 20°C and final cooling takes place in the cold store.

In large organisations, the trays (overwrapped with heat-shrink material) or cardboard boxes of packaged yoghurt are usually stacked on a wooden pallet which is later shifted using a forklift truck, that is, from production area → cold store → transport vehicle. Alternatively, metal trollies could be used, for example the Tetra-tainer type produced by the Tetra group. The packaged yoghurt in its single-trip shipping container is stacked onto these trollies. The advantage of this system is the ease of movement of the product from cold store to transport vehicle and from transport vehicle to supermarket and/or refrigerated cabinet. Such methods of handling mean that the retail cartons are not handled at all from the time that they leave the dairy until they can be picked up by the consumer.

Handling the outer or shipping container(s) can be integrated, in part, with the packaging and/or filling machines and the degree of automation is primarily governed by the throughput of the filler and the cost of labour. Manual handling of these containers is very common where the labour costs and the daily scale of production are low. Some of the devices for moving the yoghurt cups or cartons into shipping containers include: (a) a specially designed nested packaging tray (made from cardboard or plastic) holds the yoghurt pots in place and a folding flap on the upper part of the tray secures the pots in position (Galiegue and Thiry, 1990), (b) an improvement for the in-line palletising of trays of yoghurt suitable for use in situations where only manual palletising appear to be possible was described by Anon. (1987b); incidentally, this mechanical system is known as the “Somic Paletta”, (c) a system for handling blanks of rectangular cardboard which folds to form a tray-like receptacle suitable for carrying yoghurt pots in rows; this has been patented in Germany (Anon., 1987c), and (d) U-shaped tray for 250g yoghurts packs or a cardboard wrap-around for larger packs can be secured for palletising using a 2mm plastic band and antislip hot glue to replace stretch or shrink wrapping (Schlicht, 1996). Rosti (1995) has reviewed the latest trends and developments in multipacks and wrap-around assemblies, including the economics, materials and different closure systems.

2.14 Refrigerated cold storage, transport and distribution

Cooling the yoghurt to <10°C, and maintaining this low temperature until the product reaches the consumer, helps to slow down the biological and biochemical reaction(s) that are taking place in the yoghurt. The former reactions result from the metabolic activity of the yoghurt starter culture and possibly any microbial contaminants that resisted heat treatment and survived the fermentation process or were introduced as post-production contaminants (e.g. yeast and moulds). Possible biochemical reactions are:

- fat oxidation in the presence of oxygen,
- hydration of the protein constituent in yoghurt,
- changes in the colour of the fruit additive (e.g. becomes dull and pale), can take place due to acidic condition of the product,
- slight dehydration may take place and the exposed surface of the yoghurt may change its physical appearance,
- presence of added hydrocolloids (stabilisers) and/or pectins from the fruit improves the viscosity/consistency of yoghurt during storage.

In order to minimise some of these reactions, the refrigeration of yoghurt is essential and, with this proviso, the keeping quality of the product could well be up to three weeks from the date of production. However, during the first 24–48 hours of cold storage an improvement in the physical characteristics of the coagulum is observed, mainly due to the hydration and/or stabilisation of the casein micelles, and hence it may be desirable to delay the sale/distribution of the yoghurt accordingly.

Since the quality of yoghurt is dependent on a multitude of factors after production, the following recommendations may help to ensure that the product reaches the consumer in a satisfactory condition. Notably, recommendations for the hygienic manufacture of milk and other dairy products including yoghurt encompass the implementation of hazard analysis critical control point (HACCP) and/or other similar systems, and for this reason dairy products have been classified in three categories according to their temperature requirements during storage → transport and distribution → retailing (Anon., 1994c, 1995c): (a) products stored at $<-18^{\circ}\text{C}$ such as ice cream and related frozen products, (b) short shelf life perishable products (e.g. pasteurised liquid milk, cream, yoghurt, fermented milks, fresh and soft cheeses, butter and retail portions of hard and semi-hard cheeses) to be stored between 0°C and 10°C and (c), products such as UHT milk, powders, canned products and processed cheese may be stored at ambient temperature but $<30^{\circ}\text{C}$.

Therefore, it has been recommended (Anon., 1994c, 1995c) that yoghurt should be stored at $0-10^{\circ}\text{C}$ ($\pm 1^{\circ}\text{C}$ temperature tolerance) and in the same temperature range during transport, but with a $\pm 2.5^{\circ}\text{C}$ temperature tolerance. However, to safeguard the quality of the product, most large manufacturers tend to store and transport yoghurt at $<10^{\circ}\text{C}$ (Hinsperger, 1990; Farquhar and Symons, 1992).

2.14.1 The cold store

- Reduce, as far as possible, rough mechanical handling of the packaged yoghurt.
- Maintain the storage temperature as low as possible (i.e. $<5^{\circ}\text{C}$) and avoid any fluctuations.
- Provide good cold air circulation in the store, especially if the yoghurt is filled at 20°C and final cooling takes place in the cold store.
- Avoid losses of cold air through the use of a poorly designed insulated store.
- If the yoghurt is packaged in a transparent container, protect the product using special lighting to reduce decolorisation or oxidation.
- Always retain the packaged yoghurt for at least 48 hours before dispatch, so that the final stability of the coagulum is achieved.

2.14.2 During transport

- Refrigerated transport is required during the summer months in the temperate zones of the northern or southern hemispheres; during the winter months insulated lorries can be used.
- In tropical and subtropical areas, refrigeration of the transport vehicle is necessary.
- During transport, shaking the yoghurt can lead to a reduction in viscosity and whey syneresis; it is difficult to overcome this defect, especially during long road journeys.

The packaged yoghurt (i.e. unit and shipping containers) is subjected to vibratory motions during shipping and distribution and the potential damage to the product may include broken or damaged structure of set yoghurt gel, whey separation, disruption of the coagulum of stirred yoghurt and formation of a narrow skin of yoghurt between the foil laminate and the tip of the plastic cup. Richmond *et al.* (1985) studied the physical damage to set yoghurt packaged in waxed paper cartons using a vibratory table. In order to simulate the conditions during transport, 12 yoghurt cartons were placed in a cardboard nested tray, with or without stretch wrapping, and stacked 10 high. The results suggest the following:

- Yoghurts made without the addition of stabilisers had high levels of syneresis.
- Stretch wrapping minimised the effect of whey separation.
- Most damage to the yoghurt occurred in the top layer of the stack.
- Incubation and cold storage of packaging materials (i.e. unit and shipping) caused changes in physical structure which resulted in loss of stackability and product loss.

The same authors concluded that similar “challenge” tests should be conducted on yoghurt packed in different plastic cups of different designs, because the shape of the container may affect the coefficient of friction. Nonetheless, no more studies have been carried out to date.

It is worthwhile pointing out that vehicles used for transporting yoghurt should comply with special recommendations (UN, 1991) which include the installation of an automatic temperature probe (ATP) device and the requirement that trucks should have smooth internal surfaces that can be easily cleaned, be fitted with suitable shelvings, if any, and that door openings should be fitted with plastic strips to minimise heat loss.

2.14.3 The retail shop and the consumer

- The yoghurt must be displayed in refrigerated cabinets until it is purchased.
- Yoghurt should be consumed directly or otherwise stored in a domestic refrigerator until required.
- Yoghurt should be consumed around 10°C, as below this temperature the flavour profile is not appreciated due to the coldness, and above 10°C the product loses its freshness and may undergo a reduction in viscosity.

2.15 Conclusion

The quality of yoghurt (set or stirred types) is influenced by a multitude of factors during the preparation of the milk base, processing stages, packaging, storage and distribution. The following summary of points to be considered may help to ensure that a quality product reaches the consumer:

- level of protein content in the milk base
- process parameters such as homogenisation and heat treatment and on very rare occasions the addition of coagulants
- addition of stabilisers
- exopolysaccharide production by the starter cultures
- development of acidity and/or rate of acid development
- presence of inhibitory agents in milk
- post-fermentation acidification
- post-production heat treatment (refer to Chapter 5)
- vibratory motion during distribution and retailing
- mechanical handling of the coagulum (refer to Chapter 3)
- miscellaneous treatments such as the use of oils, fat substitutes or postfermentation concentration (refer to Chapter 5).

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